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Dietary intake and dental caries in children

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DIETARY INTAKE AND DENTAL CARIES IN CHILDREN

by

Oitip Chankanka

An Abstract

Of a thesis submitted in partial fulfillment
of the requirements for the Doctor of
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Thesis Supervisor: Professor Steven M. Levy

ABSTRACT

Dental caries is a common childhood disease and important health problem in the United States and throughout the world. Most studies that have assessed risk factors for dental caries focused on non-modifiable risk factors such as previous caries experience and socioeconomic status. It is also important to investigate modifiable risk factors that can be used in developing guidelines for risk assessment and prevention. The present dissertation assessed mainly the associations between dental caries and modifiable factors, including dietary factors, water fluoride levels and toothbrushing frequency in children, while adjusting for non-modifiable factors. Data were obtained from subjects who were participants in the Iowa Fluoride Study. Dietary data were collected using 3-day dietary diaries from 1.5 months to 8.5 years and detailed questionnaires from 9 years to 13 years. Dental caries examinations were conducted at about 5, 9 and 13 years of age. There are three main analyses.

The first analysis assessed risk factors for a 4 group primary dentition caries experience variable: the caries-free (reference group), the d_1, the $d_{2+}f$, and the $d_1d_{2+}f$ groups. The dietary consumption frequencies (from ages 3 to 5 years) for the children in the 4 caries groups were compared using multivariable multinomial regression analyses. Lower consumption frequency of milk at meals and greater consumption frequency of pre-sweetened cereal at meals significantly increased the likelihood of being in the d_1 group. Greater consumption frequency of regular soda pop at snacks significantly increased the likelihood of being in the $d_1d_{2+}f$ group. Greater consumption frequency of added sugars at snacks significantly increased chance to be in the $d_{2+}f$ group and the $d_1d_{2+}f$ group.

The second manuscript assessed risk factors for new mixed dentition cavitated caries determined based on surface-specific transitions from the primary to mixed dentition exams on 16 teeth using logistic regression analysis. Greater consumption frequency of processed starch at snacks significantly increased the likelihood of having new cavitated caries ($p = 0.04$ for the model excluding previous caries experience).

The third manuscript used negative binomial regression with the Generalized Linear Mixed Models procedure to assess separately the longitudinal associations of 1) new non-cavitated caries and 2) new cavitated caries with modifiable risk factors. Surface-specific counts of new non-cavitated caries and cavitated caries at each of the primary, mixed and permanent dentition examinations were used as outcome variables. Greater consumption frequency of 100% juice was significantly associated with fewer non-cavitated and fewer cavitated caries surfaces.

In this study, some factors were associated with caries at one age only, while others were associated with caries across childhood. Consumption of foods or beverages at meals generally decreased their cariogenicity. Previous caries experience is strongly associated with other independent variables in the regression models that examined risk factors for new cavitated caries. Thus modifiable factors that usually have weaker associations with caries might not be retained in the models due to collinearity issues. Future researchers are encouraged to present results both ways so that scientific communities can best interpret the complex results. Also, repeated measures analysis might be more appropriate for variables that are common in all age groups, such as toothbrushing frequency and fluoride exposures. More studies of the complex relationships between diet and caries are needed, including additional studies that place more emphasis on investigation of modifiable risk factors for both non-cavitated and cavitated caries.

Abstract Approved: __________________________________
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Iowa City, Iowa

CERTIFICATE OF APPROVAL

PH.D. THESIS

This is to certify that the Ph.D. thesis of

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has been approved by the Examining Committee for the thesis requirement for the Doctor of Philosophy degree in Oral Science at the July 2010 graduation.

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To my mother, my father, my sisters and my brothers

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Dental caries is a common childhood disease and important health problem in the United States and throughout the world. Most studies that have assessed risk factors for dental caries focused on non-modifiable risk factors such as previous caries experience and socioeconomic status. It is also important to investigate modifiable risk factors that can be used in developing guidelines for risk assessment and prevention. The present dissertation assessed mainly the associations between dental caries and modifiable factors, including dietary factors, water fluoride levels and toothbrushing frequency in children, while adjusting for non-modifiable factors. Data were obtained from subjects who were participants in the Iowa Fluoride Study. Dietary data were collected using 3-day dietary diaries from 1.5 months to 8.5 years and detailed questionnaires from 9 years to 13 years. Dental caries examinations were conducted at about 5, 9 and 13 years of age. There are three main analyses.

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In this study, some factors were associated with caries at one age only, while others were associated with caries across childhood. Consumption of foods or beverages at meals generally decreased their cariogenicity. Previous caries experience is strongly associated with other independent variables in the regression models that examined risk factors for new cavitated caries. Thus modifiable factors that usually have weaker associations with caries might not be retained in the models due to collinearity issues. Future researchers are encouraged to present results both ways so that scientific communities can best interpret the complex results. Also, repeated measures analysis might be more appropriate for variables that are common in all age groups, such as toothbrushing frequency and fluoride exposures. More studies of the complex relationships between diet and caries are needed, including additional studies that place more emphasis on investigation of modifiable risk factors for both non-cavitated and cavitated caries.

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CHAPTER I

INTRODUCTION

Dental caries is a very common and important health problem for children. Caries is five times as common as asthma and, thus, the most common chronic disease of childhood. Even in low caries risk populations, caries is still a very common childhood disease. The three essential factors for caries development are dental plaque (which can contain harmful bacteria), fermentable carbohydrate from the diet and susceptible tooth. Approaches to prevent dental caries include reducing dental plaque levels, changing the bacterial composition of plaque, and changing dietary intake patterns and frequency. In addition, fluorides are very effective in preventing dental caries, including fluoride toothpaste, water fluoridation, fluoride mouthrinse and professional topical fluoride application, primarily by inhibiting mineral loss from the tooth.

Children of low socio-economic status (SES) have higher caries risk than those of middle or high SES and are usually a priority group in dental research and prevention. However, those in all the other groups are also susceptible to dental caries. Additionally, children of moderate caries risk outnumber those who are of higher caries risk. There are a limited number of studies that have assessed caries risk factors in such population with lower caries rates. Studies assessing the relationships between dental caries and dietary intake in these populations are needed.

Based on previous studies which have examined the relationships between dental caries and diet, it is not possible to draw simple, clear conclusions about these complex relationships. This is largely because of limitations and/or differences in the methods used. Thus, there are conflicting results regarding the relationships between dietary variables and dental caries status. For example, intake of carbohydrates was positively associated with higher dental caries increment in some studies (Beighton *et al.*, 1996; Kwon *et al.*, 1997; Sohn *et al.*, 2006), while no relationship was found in others (Bruno-

Ambrosius *et al.*, 2005; Heller *et al.*, 2001; Richardson *et al.*, 1977). The different outcomes of these studies are probably due to different designs, different populations, different definitions of dependent variables of caries status, different definitions of "diet", different fluoridation status, and other factors. Thus, it is difficult to draw firm conclusions from the previous studies and to make recommendations regarding dietary modifications to prevent dental caries.

Obviously, non-cavitated caries is currently and has been an important theme in dental research this decade. Non-cavitated caries is an early stage of the dental caries process which is also a reversible phase of the disease. Non-cavitated caries does not need restorative treatment, but may be amenable to preventive procedures. Non-cavitated caries has been mentioned in many articles; however, few of the published studies have examined the relationships between diet and non-cavitated caries status by itself or a weighted outcome combining non-cavitated caries relative to cavitated caries. Most studies only have assessed the effects of dietary factors on (frank) cavitated caries. Few studies assessed the association between overall caries (combined non-cavitated caries with cavitated caries) and diet.

Therefore, the overall goal of this doctoral research dissertation is to examine longitudinally the effects of dietary variables, including frequency and/or amount of food and beverage intakes, on caries status. Specifically, the research will assess caries prevalence and incidence for non-cavitated and cavitated caries outcomes. First, analysis focused on examination of dietary risk factors for age 5 non-cavitated caries, as well as cavitated caries, after adjusting for other caries-related factors. The purpose of the second analysis was to examine dietary risk factors for children having new cavitated caries between ages 5 and 9 years, after adjusting for other caries-related factors. The third major analysis assessed the longitudinal associations between caries outcomes and dietary risk factors, after adjusting for other non-dietary factors. The study children are members of the Iowa Fluoride Study cohort which has been followed since birth.

Detailed dietary data concerning cohort children were collected starting at age 1.5 months and every 1.5 to 6 months thereafter. These children are mostly residents of Iowa and are generally from moderate to high SES families and thus, as a group, assumed to be low-to-moderate caries risk children.

The results of this study will be useful in guiding recommendations for dietary modifications to prevent dental caries in these age groups. The results may be especially useful as reference information concerning the relationships between diet and dental caries in a low-to-moderate risk population.

CHAPTER II

LITERATURE REVIEW

General Concepts

Even though dental caries prevalence generally has declined in the U.S. and most westernized nations during the past few decades, it is still a common childhood disease worldwide and in the U.S. From the U.S. National Health and Nutrition Examination Surveys (1988-94 and 1999-2004), among children 6-8 and 9-11 years of age, prevalence of dental caries in the permanent dentition decreased from 14.5% and 35.9% to 10.2% and 31.4%, respectively (Dye et al., 2007). For adolescents, prevalence of dental caries in permanent dentition teeth among adolescents 12-15 years of age from the NHANES data decreased from 57% (1988-1994) to 51% (1999-2004). However, dental caries prevalence in the primary dentition increased from 24.2% to 27.9% for children aged 2 to 5 years old, and from 49.9% to 51.2% for children age 6-11 years old (Dye et al., 2007).

As demonstrated by the NHANES data, dental caries experience in the primary and permanent dentitions increases dramatically with age. Caries prevalence of children in Iowa generally has followed the same age-related trend as the NHANES III data. The statewide oral health survey of Iowa children was conducted in 1999 with 1,713 children aged 6 to 10 years old. The study results showed that the number of caries-free children at 7 years old (84.4 percent) was almost 20 percent higher than the number of caries-free children at 10 years old (67.1 percent) (Arjes, 2001).

Although overall caries prevalence has declined, the current status of dental caries is still problematic. As reported in the U.S. Surgeon General's Report on Oral Health, among children age 5 to 17 years, 43.6 percent of poor children and 23.4 percent of non-poor children had one or more untreated decayed primary and/or permanent teeth (Oral Health in America: A Report of the Surgeon General, 2000). The results of the Iowa Oral Health Survey for children in the age range from 7 to 10 years old showed that 20.3

percent of children age 10 years old had untreated caries (Arjes, 2001). Dental caries has consequences in functional, psychological, and economical dimensions related to quality of life. The Surgeon General's Report on Oral Health (Oral Health in America: A Report of the Surgeon General, 2000) also addressed the relationships of oral health with quality of life, in that oral health affects diet, nutrition, sleep, psychological status, social interactions, education, and career development. The report also emphasizes that having missing teeth is linked to qualitatively poorer diet. The Surgeon General's Report also presented the indirect economic costs of oral disease: the number of school days lost for youths 5 to 17 years of age was 3.1 days per 100 persons per year. In addition, national expenditures for dental services in the United States in 1998 were 58.8 billion dollars (Oral Health in America: A Report of the Surgeon General, 2000).

Dental Caries

Dental caries is an infectious disease that destroys tooth structure. The caries process starts when oral bacteria (e.g., *Streptococcus Mutans*) in dental plaque produce acid by metabolizing dietary carbohydrates. Direct exposure of the tooth to the acid over a period of time results in dissolution of the tooth's mineral, a process called demineralization, which is the initial stage of the caries process. This demineralization usually occurs if the oral cavity does not have sufficient defensive mechanisms to protect enamel from the detrimental effects of frequent acid attacks. The loss of mineral from tooth structure causes microporous areas which are identified as white spots. If the loss of mineral from tooth structure continues, a cavity develops.

The factors which are often considered to most significantly affect the dental caries process are diet and fluoride exposures. Theoretically, diet and nutrition might affect the dynamic process of tooth demineralization and remineralization in many ways. Sugars in diet are a substrate for bacteria in dental plaque. The low pH of acid produced by bacteria supports the growth of the acidogenic and aciduric bacteria. In contrast, a diet lower in sugars and high in calcium may favor remineralization. In addition, theoretically, the presence of fluoride in the oral cavity enhances remineralization by reducing the critical pH (Touger-Decker and van Loveren, 2003).

Some theories regarding dental caries are mostly obtained from *in vitro* and animal models. Dr. Ole Fejerskov, an expert on dental caries, pointed out that so-called "necessary causes" concerning caries are primarily derived from *in vitro* and animal models where strict control of confounding factors can be accomplished (Fejerskov, 1997). Such studies are not a component of Evidence Based Dentistry. However, the environment of the human oral cavity is subjected to widely different and variable exposures within and among individuals. Some authors quote "known established facts" that are often derived from numerous reviews of the published studies, which might not be valid for the current discussion. Thus, clinical studies need to be performed based on the information from basic science research regarding dental caries. Such studies can be used to develop more effective prevention of dental caries (Fejerskov, 1997).

Cavitated Caries and Non-Cavitated Caries

Traditionally, studies concerning the factors influencing caries initiation and caries progression analyze a primary outcome variable as the total change from sound to frankly cavitated teeth or tooth surfaces during a defined period of observation. From knowledge of the caries process, demineralization will first appear clinically as a white spot lesion. The transition from sound tooth surface to non-cavitated caries lesion (white spot) sometimes is overlooked. The transition from sound tooth surface to non-cavitated caries indicates higher caries risk compare to those sites that stay sound over time. Thus, the presence of non-cavitated caries might be a more sensitive indicator to evaluate factors associated with caries and disease progression. Especially in lower-risk populations, non-cavitated caries as the outcome variable might be a good indicator with greater sensitivity to differentiate higher risk from lower risk.

The detection of non-cavitated caries lesions has become a very important and necessary topic as a prerequisite for preventing cavitated caries. Thus, a major goal of dental caries management is to arrest the disease without the need for restorative treatment (Thylstrup, 1989).

<u>Caries Preventive Methods</u>

To prevent caries development and inhibit and reverse non-cavitated caries from developing into frank cavitation, the process of demineralization must be stopped. The main methods are to remove cariogenic bacteria from the tooth surface (by oral hygiene care or using chemical agents), to change/modify diet and/or to use fluoride and/or other remineralizing or preventive agents. There are many studies regarding use of chemical agents and fluoride to inhibit the caries process. However, such studies are costly and are often conducted only with high-risk populations. Many studies concluded that chlorhexidine is effective at reducing the number of *Mutans Streptococci* in caries-active or high-risk patients (Achong *et al.*, 1999; Splieth *et al.*, 2000). Moreover, these methods have been found to be less effective in low or moderate caries-risk populations (Fennis-le *et al.*, 1998). Recently, several studies have reported non-significant associations between caries increment/incidence/prevalence and fluoride usage in low-risk populations (Frame *et al.*, 2000; van Rijkom *et al.*, 2004). Changes in the diet are one of the possible methods to prevent dental caries, and it also may be a good measure for prevention of dental caries in the moderate-caries risk population.

Relationship of Diet and Dental Caries

The interest in dietary effects on dental caries has existed for more than 50 years. The famous "Vipeholm Dental Caries Study" (Gustafsson *et al.*, 1954) has been mentioned numerous times in dental research and education. This study documented the increased caries incidence in subjects with a high sugar diet during and between meals. More recently, many studies have tried to examine the effect of diet on dental caries in everyday life in the general population. Although many researchers have examined several aspects of this relationship using various designs, there are controversies. Most studies that have examined the relationships between diet and caries in humans of defined age and socio-demographic group. Different age groups may have different risk factors and outcomes according to the populations' specific characteristics. Some results of studies in specific age groups might not be generalized to other age groups. Additionally, most studies included only non-modifiable factors, such as previous caries experience, age, and socioeconomic status as the studies' independent variables. There are small numbers of studies that examined the association of modifiable factors, such as dietary intake frequencies, drinking water fluoride levels, and toothbrushing frequencies, with dental caries in each age group. Moreover, the results from this limited number of studies are somewhat inconclusive. Thus, there is no firm agreement regarding the precise details of the relationship between dental caries and diet.

Preschool Children

Non-Cavitated and Cavitated Caries in the Primary Dentition

There are a limited number of studies reporting non-cavitated caries in the primary dentition. There are no published studies at all before 1990 reporting non-cavitated caries prevalence or incidence in the primary dentition. In the last two decades, the concept of minimal intervention and the attempt to treat caries as a disease at an early stage have driven the need for studies concerning non-cavitated caries in developed countries.

There are 6 published studies (Autio-Gold and Tomar, 2005; Bankel et al., 2006; Carvalho et al., 1998; Holbrook et al., 1995; Kolker et al., 2007; Ramos-Gomez et al., 2002) where investigators diagnosed non-cavitated caries (initial caries) in preschool children, four of them (Autio-Gold and Tomar, 2005; Bankel et al., 2006; Holbrook et al., 1995; Ramos-Gomez et al., 2002) reported separately the mean numbers of cavitated and non-cavitated caries lesions, one (Carvalho et al., 1998) reported the mean number of non-cavitated and cavitated lesions together and one study (Kolker et al., 2007) reported only the distribution of children by number of combined caries lesions in the ranges of 0, 1-4, 5-11, and 12-52 surfaces. The first study published in 1995 (Holbrook *et al.*, 1995) reported that 77% of 43 Swedish children aged 5 years old had cavitated and/or non-cavitated caries. The mean dmfs (only cavitated caries) score and count of surfaces with initial caries score were 7.1 and 2.6, respectively. The mean number of surfaces with initial caries in this study was relatively small because they examined non-cavitated lesions only on smooth surfaces (excluded pits and fissures). The second study (Carvalho *et al.*, 1998) described non-cavitated caries and cavitated caries in Belgian 3- to 5-year-old children. This study reported non-cavitated caries (active) lesions only as part of the defs scores. The mean defs scores (including cavitated and non-cavitated caries) were 2.04, 2.46 and 3.75 for children ages 3, 4 and 5, respectively. Non-cavitated caries lesions represented about half of all lesions (presented in a graph without actual numbers in a table). The third study (Ramos-Gomez *et al.*, 2002) reported the mean numbers of ds, dfs, and idfs (initial caries and cavitated caries combined). They examined 146 children aged 3 to 55 months from low-income families in San Francisco. They reported that 43% of these children had at least one obvious carious lesion and 16% had only incipient lesions. Overall, mean dfs and idfs for these children were 2.2 and 3.1, respectively. The study conducted in Florida (Autio-Gold and Tomar, 2005) reported that 86% of 221 children 5-6 years of age had cavitated and/or non-cavitated caries. The mean numbers of active non-cavitated caries and cavitated caries surfaces were 2.91 and 2.52, respectively. A Swedish study (Bankel *et al.*, 2006) examined 221 2- to 3-year-old children. They reported that prevalence of children with manifest caries and with initial caries were 7.2% and 11.3%, respectively. The mean numbers of defs (cavitated caries only) in 2-year-olds and 3-year-olds were 0.2 and 0.6, respectively, and the mean numbers of initial caries in 2-year-olds and 3-year-olds were 0.1 and 0.6, respectively.

Among the available studies, one study (Bankel *et al.*, 2006) found that the percentage of children with only non-cavitated (initial) caries (11.3%) was higher than the percentage of children with cavitated caries (7.2%), while another study (Ramos-Gomez *et al.*, 2002) with much higher disease rates found that there were more children with cavitated caries (43.2%) than those with non-cavitated caries only (15.8%). For children aged 5-6 years old, more than 75% had non-cavitated and/or cavitated caries (Autio-Gold and Tomar, 2005; Holbrook *et al.*, 1995). Most studies found that non-cavitated caries represented more than 50% of all lesions (Autio-Gold and Tomar, 2005; Bankel *et al.*, 2006; Carvalho *et al.*, 1998).

Non-Cavitated Caries and Dietary Factors

One study (Ramos-Gomez *et al.*, 2002) grouped the children into 1) children with at least one cavitated lesion, 2) children with initial caries only and 3) caries-free children, and reported descriptive information—demographic characteristics, diet and feeding practices, access to care and bacterial level related to the three groups. However, the authors assessed only the associations between cavitated caries and associated factors. They reported no significant differences in the diet and feeding practices of children with at least one cavitated lesion and caries-free children. There are three studies (Bankel et al., 2006; Holbrook et al., 1995; Kolker et al., 2007) that combined non-cavitated caries with cavitated caries for analytical purposes, thus assessing the associations between overall caries and related factors. These studies found significant associations between frequent sugar consumption, misusing sugar (defined as having sugar four or more times on the previous working day or having two or more between meal snacks) or frequent sugared drinks consumption and overall caries. However, none of these three studies examined the relationships between dietary factors and non-cavitated caries individually or weighted non-cavitated caries relative to cavitated caries. Thus, there are no published studies that have assessed relationships of non-cavitated caries with associated risk factors.

Cavitated Caries and Dietary Factors

There are only a few cohort studies (Holbrook et al., 1995; Marshall et al., 2005; Ohlund et al., 2007; Ollila and Larmas, 2007; Thitasomakul et al., 2009) that have examined caries risk factors for cavitated caries in the primary dentition. More cross-sectional studies (Al-Malik et al., 2001; Bankel et al., 2006; Gibson and Williams, 1999; Nunn et al., 2009; Sayegh et al., 2005; Sohn et al., 2006; Tsai et al., 2006; Vignarajah and Williams, 1992) assessed the associations between cavitated caries and caries-related factors.

Holbrook and colleagues (Holbrook et al., 1995) investigated caries risk factors in 43 children at age 5 years at baseline and 15 months later. Greater intake frequencies of sweets and cariogenic between-meal snacks were associated with cavitated caries in this study. Sayegh et al. (Sayegh et al., 2005) assessed caries risk factors in 1075 4- 5-year-old Jordanian children. They conducted a multivariable logistic regression and reported that confectionery consumption was associated with significantly increased risk for presence of cavitated caries, after adjusting for other caries-related variables. Gibson and Williams (Gibson and Williams, 1999) examined cavitated caries risk factors in children aged 1.5-4.5 years. They reported that frequency of sugar confectionery consumption was associated with cavitated caries only among children who brushed their teeth less than twice a day. Tsai and colleagues (Tsai et al., 2006) conducted a study to investigate etiological factors for cavitated caries in 2-to 6-year-old children. They reported that cavitated caries was strongly associated with consumption of sweets in the multivariable logistic regression. These studies reported positive associations between sweets/confectionery consumptions and cavitated dental caries. One cohort study

(Ohlund et al., 2007) examined and reported no association between intake of candies/sugar and cavitated caries in 4-year-old children with low caries prevalence. One cross-sectional study (Campus et al., 2007) reported no significant association between frequency of sweet food consumption and caries in 55 2.5- 4.5-year-old children.

Most of the studies (Al-Malik et al., 2001; Marshall et al., 2005; Sohn et al., 2006) that examined the association between carbonated drink intake and cavitated caries reported a significant association between them. One cohort study (Ohlund et al., 2007) did not find a significant association between consumption of soft drinks and cavitated caries in 4-year-old children. However, many of these studies were done on non-U.S. populations, with substantially different diet their diets differ from our. Thus, their data are not necessarily generalizable to the United States.

For the primary dentition, intakes of sweets/confectionery and carbonated drinks were significant risk factors for cavitated caries.

Elementary School-Aged

Non-Cavitated and Cavitated Caries in the Mixed Dentition

Dental caries in the mixed dentition has been reported to be more frequent than dental caries in younger children. However, there are only a limited number of studies that have assessed and reported non-cavitated caries prevalence/counts. In 1992, Ismail and his team (Ismail et al., 1992) conducted a cross-sectional study to describe the prevalence of non-cavitated and cavitated caries in 911 7- 9-year-old children in Montreal. They reported more non-cavitated caries lesions than cavitated caries lesions in this population. About 62% of these children had cavitated caries and 78% had cavitated and/or incipient caries. Skeie and colleagues (Skeie et al., 2004) conducted a cohort study to explore caries development at age 5 and at 5 years later. There were 186 children at the follow-up examination visits. They reported that the mean non-surface-specific increment (for both non-cavitated and cavitated caries excluding incisors) was 3.05. The mean number of initial lesions increment was slightly higher than that of manifest lesions increment.

Poulsen and Malling Pedersen (Poulsen and Malling Pedersen, 2002) reported on national data (1988-2001) concerning dental caries for Danish children. The data did not show the prevalence of dental caries. However, they reported that 7-year-old children in 2001 (mean DMFS of 0.15) had 63% fewer surfaces with cavitated caries compared with 7-year-old children in 1988 (mean DMFS of 0.41). One study (Yabao et al., 2005) reported separately prevalence for the primary and permanent teeth in the mixed dentition. They reported that 6-12-year-old children had slightly higher cavitated caries prevalence in primary teeth (72%) than in permanent teeth (68%) and they had a much greater mean number of affected primary teeth (4.1 dmft) than permanent teeth (2.4 DMFT).

One study (Mattila et al., 2001) determined caries increment for 1059 children from the age of 7 to the age of 10. The mean cavitated caries increment was 0.45 during the 3 years period. Caries increment of the primary teeth, permanent teeth and combined primary and permanent teeth were found in 24%, 25% and 36% of children, respectively. Zhang and van Palenstein Helderman (Zhang and van Palenstein Helderman, 2006) conducted a cohort study in 6- 7-year-old children at baseline and two years later. They reported that cavitated caries prevalence of primary teeth was 77% at baseline (no data reported for the follow-up) and cavitated caries prevalence of permanent teeth at baseline and at the follow-up were 6% and 29%, respectively. At both exams, teeth with enamel caries were more common than teeth with dentin caries. Another cohort study (Scheutz et al., 2007) in children (mean age of 7.6) reported 21% of these children developed new caries at both the first and second 3-year-follow-up periods. Mean caries increments were 0.27 and 0.80 (for all children) at the first and the second follow-up periods, respectively.

Caries Incidence and Associated Factors

Previous Caries Experience

Most studies (Leroy et al., 2005; Peretz et al., 2003; Skeie et al., 2004; Steiner et al., 1992; Tagliaferro et al., 2008; Vallejos-Sanchez et al., 2006; Vanobbergen et al., 2001; Zhang and van Palenstein Helderman, 2006) that reported cavitated caries incidence/increment in primary school-age children examined the association between previous caries experience and new cavitated caries occurrence. They consistently found a strong and significant association between previous caries experience and new caries.

Dietary Factors

There are fewer studies (Leroy et al., 2005; Mattila et al., 2001; Tagliaferro et al., 2008; Vanobbergen et al., 2001) that have examined the associations between cavitated caries incidence and modifiable factors, such as dietary intakes, fluoride usage and toothbrushing frequency. Some studies (Mattila et al., 2001; Vanobbergen et al., 2001) found significant associations between consumption of sweets/sweetened drinks and new cavitated caries.

Mattila and colleagues (Mattila et al., 2001) conducted a cohort study with 1,059 children to investigate the association between caries increment from age 7 to 10 years and risk factors. They found that consumption of sweets more than once a week significantly increased the chances of having caries increment in permanent teeth, but not in primary teeth. One study (Vanobbergen et al., 2001) assessed caries risk factors for the mixed dentition. Children were examined for dental caries at baseline (age 7) and at age 10. This study reported that the daily use of sugar-containing drinks between meals increased the risk of having cavitated caries increment.

Other studies (Leroy et al., 2005; Tagliaferro et al., 2008) reported no significant associations between dietary consumption (sweets, sugar or snacks) and new cavitated caries. Leroy and colleagues (Leroy et al., 2005) examined the risk factors for incidence of cavitated caries in permanent first molars in a 6-year-follow-up study (from age 6 to 12 years). The multivariate survival analysis revealed no significant associations between consumptions of sweets, sugar-containing drinks or biscuits and new caries. Another study (Tagliaferro et al., 2008) assessed the risk factors for high caries increment children. They followed children age 6 to 8 years for 7 years. Numbers of between-meal snacks and frequency of sugar consumption were not significantly associated with high caries increment.

In this age group, we cannot draw a strong conclusion between dietary factors and caries increment.

Adolescents

Non-Cavitated and Cavitated Caries

There are only a few non-experimental studies that have reported prevalence and/or counts of non-cavitated/initial caries and cavitated caries in the early permanent dentition. Some studies (Alm et al., 2007; Gustafsson et al., 2000) examined approximal caries only in posterior teeth using only bitewing radiographs. The cross-sectional study conducted in Sweden by Alm et al. (Alm et al., 2007) reported that 66% of these children had approximal caries. Mean numbers of approximal initial and manifest caries in posterior teeth recorded on bitewing radiographs of 15-year-old children were 2.78 and 0.45 lesions, respectively. Gustafsson and colleagues (Gustafsson et al., 2000) conducted a retrospective study to determined the number of initial caries lesions and manifest caries lesions in 14- to 19-year-old Swedish adolescents using bitewing radiographic examinations. At age 15, the girls had means of 5.5 non-cavitated caries lesions and 1.0 cavitated caries lesions and the boys had means of 4.9 non-cavitated caries lesions and 0.6 cavitated caries lesions.

Other studies (Amarante et al., 1998; Machiulskiene et al., 1998; Weissenbach et al., 1995) conducted either clinical examinations only or clinical and bitewing radiograph examinations for dental caries prevalence/counts. Amarante et al. (Amarante et al., 1998) conducted both clinical and bitewing radiographic examinations in Norway and reported that the prevalence of initial and/or manifest caries was 63% and 98% for 12- and 18-year-old children, respectively. Children ages 12 and 18 had means of 6.9 and 10.9 non-cavitated caries surfaces and 0.8 and 1.7 cavitated caries surfaces, respectively. Madchiulskiene and colleagues (Machiulskiene et al., 1998) examined 9- to 14-year-old Lithuanian children using the Nyvad criteria (Nyvad et al., 1999). They reported that caries prevalence was more than 99%, where including all categories of caries. They reported means of active non-cavitated and inactive non-cavitated caries of 4.9 and 4.4 surfaces, respectively. Mean active cavitated and inactive cavitated caries were 2.9 and 0.6 surfaces, respectively.

Thus, for this age group overall in the few studies available, dental caries was very common and more than two-thirds of the affected surfaces were non-cavitated/initial caries compared to less than one-third cavitated/manifest caries.

Dental Caries and Associated Factors

Among these studies that reported both non-cavitated and cavitated caries, only two studies assessed risk factors for dental caries. One study (Alm et al., 2008) examined the associations of combined counts of cavitated and non-cavitated caries at age 15 with oral hygiene and parent-related factors at ages 1 and 3 years. They reported that use of fluoride toothpaste less than twice a day at age 3 and self-reported estimate of oral hygiene care as “less good to poor” (vs. very good) increased risk of having 8 or more affected surfaces. The other cross-sectional study (Weissenbach et al., 1995) assessed the associations of initial caries lesions and DMFS with toothbrushing frequency, sugar consumption and salivary components in 12- to 14-year-old children. They found that age, salivary mutans streptococci and toothbrushing frequency were significantly associated with initial caries lesions, but consumption frequency of sweet drinks during meals and the global sugar score were not associated with initial caries lesions. They also reported significant associations between DMFS and age and salivary mutans streptococci, however, they did not find significant associations of DMFS with consumption frequency of sweet drinks during meals or with toothbrushing frequency.

For studies that investigated risk factors for only cavitated caries, there are a few that examined the associations between dietary intake factors and cavitated caries. Burt and colleagues (Burt et al., 1988) conducted a cohort study to examine the relationships between caries increment and consumption of sugars in 11- to 15-year-old children residing in non-fluoridated communities in the United States. They found that intake frequency of sugary snacks which defined as having 15% or more of sugars at baseline was not significantly associated with cavitated caries increment over the three years, but it was significantly associated with approximal caries increment (p=0.05). One cross-sectional study (Kwon et al., 1997) assessed the association between oral hygiene and dietary factors and dental caries in 12-13-year-old children in Korea. They did not find any significant associations between cavitated caries experience (yes/no) and the following variables: toothbrushing frequency, fluoride mouthrinse experience, regularity of dental check-up and between meal intake frequencies.

In summary, there is very limited information for caries risk factors for non-cavitated caries in adolescence. There are more studies that have assessed caries risk factors for cavitated caries, however, only a few studies examined the associations between dietary factors and cavitated caries. The results from these studies are different. Therefore, clear conclusions cannot be drawn from these limited and inconsistent findings.

Summary

Dental caries has been a common disease in children. There are only a few studies that have included non-cavitated caries, with most of them recently. Non-cavitated caries accounted for more than half of the total affected lesions in most studies (that included non-cavitated and cavitated caries). Non-cavitated caries can be remineralized or progress to cavitated caries lesions. There is very limited knowledge about risk factors for non-cavitated caries. Studies that examined risk factors for cavitated caries used different designs, study methods and analytical approaches. Moreover, there are inconsistent results concerning the associations between dietary intake categories and dental caries, with different studies often finding different variable significantly related vs. not. More studies that examine also non-cavitated caries risk factors are needed. Moreover, standard dietary intake categories for assessing risk factors for dental caries are needed to increase the possibility of comparing and summarizing findings from these studies.

CHAPTER III

GENERAL MATERIALS AND METHODS

Overview

As described in Chapter II, there is evidence that diet plays a significant role in caries development, but there is insufficient understanding of diet's role in cavitated caries, particularly for low-to-moderate-risk children who have been exposed to several sources of fluoride. Moreover, there is limited knowledge available relating to diet and non-cavitated caries. Thus, to better understand the relationships between diet and dental caries status, and the effects of diet on dental caries progression, this dissertation examined the associations between dental caries and dietary data of children participating in the Iowa Fluoride Study (IFS). The IFS study used 3-day food and beverage diaries every 1.5 to 6 months from the time the children were 1.5 months old until 8.5 years old. IFS questionnaires have been used to collect beverage consumptions since the age of 1.5 months. Dental caries data were obtained through standardized examinations conducted at about 5, 9 and 13 years of age. This chapter describes the IFS population and IFS data collection methods in general, as well as the results from descriptive analyses regarding diet and dental caries variables.

Study Population and Data Collection

Study Population

The Iowa Fluoride Study (IFS) recruited mothers with newborns from eight Iowa hospital post-partum wards from March 1992 to February 1995 and these children have been longitudinally followed since birth. Informed consent was obtained before collecting baseline data from mothers about their age, education, family income, and number of children in the household (Levy et al., 2001).

Data Collection

The main IFS questionnaires were sent to the mothers at 1.5-6 month intervals when the children were 1.5 months until 8.5 years old (at ages 3, 6, 9, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 54, 60, 66, 72, 84, 96, 102, and 108 months old). Until 8.5 years of age, 3-day food and beverage diaries were sent at the same time as the questionnaires. At 9 years of age, a new detailed questionnaire was developed by modifying the main questionnaire to have more details on dietary intake information, since the dietary diary was discontinued. The new questionnaires have been sent to parents every 6 months since age 9 years. The dental examinations were conducted when the children were approximately 5, 9 and 13 years old.

Collection of Dietary Data

3-Day Food and Beverage Diaries

The 3-day food and beverage diaries were mailed to the parents of study children beginning at ages 6 weeks, followed by 3 months, and then every 3 to 6 months (until 102 months old). The diaries instructed parents (or caregivers) to record all the foods and beverages that their children ate or drank for 3 days: 2 weekdays (including at least 1 childcare day, if applicable) and 1 weekend day. The diaries asked the parent to report seven items regarding each food or beverage: time (a.m. or p.m.): place (home, child care, or out), foods and beverages, brand name, type and/or preparation, water from (home, child care, out or bottled) and amount ate or drank (Marshall et al., 2005).

Using the diet abstraction worksheet, the diaries were abstracted by a trained registered dietician or diet technician. Foods and beverages consumed within a 30-minute interval counted as a single eating event. The eating events then were categorized into meals or snacks based on the time of consumption and nature of the food. Then each food or beverage item was categorized based on nature of the food and carbohydrate compositions. Items were counted as number of eating or drinking occasions at meals or between meals (snacks) for each category of food or beverage. Consumption of two servings of the same beverage or food within an eating event was counted as one occasion. Quantities of beverage intakes, but not food intakes, were also recorded in ounces. From these data, the numbers of eating occasions and ounces for these 3 days were averaged to determine the numbers of eating occasions and ounces per day.

Collection of Dental Caries Data

Dental Examination

The children were examined for dental caries. The first dental examinations were conducted when the children were about 5 years old. The second and third examinations were done at about 9 and 13 years old, respectively. Examinations were performed using a portable chair and halogen head light by trained and calibrated examiners. The teeth were dried and a DenLite® mirror (Welsh-Allyn Medical Producted, Inc., Skaneatele Falls, NY) was used for improved lighting and transilumination. The examination was conducted primarily under visualization without radiographs; however, questionable conditions were confirmed by gentle explorer use.

Diagnostic Criteria

The diagnostic criteria system used in this study (Warren et al., 2002) was a d_1/D_1-d_3/D_3 system modified from the criteria of Pitts (Pitts and Fyffe, 1988; Pitts, 1997). The criteria used in this study did not differentiate cavitated enamel (d_2/D_2) and dentine lesions (d_3/D_3), so these lesions were categorized as d_{2+} (primary teeth) or D_{2+} (permanent teeth) lesions. The d_1 or D_1 was used to categorize non-cavitated lesions, which is similar to D_1 used by others (Warren et al., 2002).

The occurrence of new caries in the second (Chapter V) and third (Chapter VI) manuscripts was determined at the surface-level. The transitions of dental caries were "surface-specific" transitions.

Collection of Other Related Factors

Baseline information concerning family income, mother's education, father's education, and number of children in the family was collected at the recruitment.

The other related factors, for example, brushing frequency and water fluoride level, were collected longitudinally using the questionnaires. Composite water fluoride levels were determined from all available public water documentation or assay of all sources of water (i.e., bottled/filtered/tap water) at each time point for each child.

Analytical Approaches

All dental caries was recorded and used at surface specific level. Non-cavitated caries and cavitated caries were considered separately. There are 3 main analyses that are presented in Chapters IV, V and VI. They had varied sample sizes. The three analyses all used the regression analysis. However, they used different types of the regression modeling procedures which were because of the different purposes and data used in each analysis. All analyses in this dissertation were done in SAS.

CHAPTER IV

THE ASSOCIATIONS BETWEEN DIETARY CONSUMPTIONS FROM 36 TO 60 MONTHS OF AGE AND PRIMARY DENTITION NON-CAVITATED CARIES AND CAVITATED CARIES

Abstract

Non-cavitated (d_1) caries is often the first visible sign of dental caries and can progress to frank decay, but may become arrested. Better understanding of the risk factors for non-cavitated lesions before they need treatment is important. Objectives: Our objective was to examine risk factors for non-cavitated caries, as well as cavitated caries. Methods: Subjects were participants in the Iowa Fluoride Study cohort. Dietary data were collected at 36, 48 and 60 months old using 3-day dietary diaries, and a dental examination was conducted at about 5 years of age. We compared the frequencies of dietary intakes of three groups:1) children having only $d_1$caries (the d_1 group, n=41), 2) children having only cavitated (d_{2+}f) caries (the d_{2+}f group, n=46) and 3) children having both $d_1$and d_{2+}f caries (the d_1d_{2+}f group, n=49) with a forth group 4) those of caries-free children (n=257). Results: Multinomial logistic regression was used, where the categorical outcome was based on the 4 caries groups, and the caries-free group was designated as the reference. In the final model, 7 variables were associated with the caries outcome. Lower milk consumption frequency at meals and greater pre-sweetened cereal consumption frequency at meals were significantly associated with a greater likelihood of being in the d_1 group. Greater regular soda pop consumption frequency and greater added sugar consumption frequency at snacks were significantly associated with being in the cavitated caries (d_{2+}f and/or d_1d_{2+}f) groups. Lower socioeconomic status (SES) and less frequent toothbrushing increased the likelihood of being in the d_1 group. Conclusions: The results of the present study suggest that different food and beverage categories are associated with being in the $d_1$group compared to the cavitated caries

groups. More frequent toothbrushing, greater milk consumption at meals and avoiding pre-sweetened cereal consumption at meals might reduce the risk of developing non-cavitated caries.

Introduction

Management of dental caries in the primary dentition is considered difficult and can involve expensive treatment due to limited child cooperation. Prevention of dental caries in preschoolers has positive benefits on both the primary and permanent dentitions. A better understanding of caries initiation and progression is necessary for more successful caries prevention.

Non-cavitated caries is considered an early stage of the dental caries process. Thus, both non-cavitated and cavitated caries are assumed to have the same risk and protective factors. However, Mascarenhas (Mascarenhas, 1998) has mentioned that the risk factors for enamel and dentin caries may not be the same.

Non-cavitated caries can be remineralized and not progress to cavitated caries lesions. Better understanding of non-cavitated caries risk factors could help us to better understand the overall caries process and lead to the development of improved interventions that could decrease risk for non-cavitated caries. Hopefully, this would eventually decrease the number of cavitated caries lesions, thus reducing the need for restorative treatment at both the individual and community levels. Monetary costs, as well as physical costs, such as pain, anxiety, and missed school, would be reduced.

Few cohort studies (Holbrook et al., 1995; Marshall et al., 2005; Ohlund et al., 2007; Ollila and Larmas, 2007; Thitasomakul et al., 2009) have assessed associations between dietary intakes and dental caries in the primary dentition. The numbers of cross-sectional studies (Al-Malik et al., 2001; Bankel et al., 2006; Gibson and Williams, 1999; Nunn et al., 2009; Sayegh et al., 2005; Sohn et al., 2006; Tsai et al., 2006; Vignarajah and Williams, 1992) and case-control studies (Mariri et al., 2003; Nobre dos Santos et al.,

2002) are also limited. A review of the English-language literature describing studies of preschoolers showed that most studies investigated associations between cavitated caries experience and specific types of diet. Some studies reported positive associations between confectionary/candy consumption and cavitated caries (Gibson and Williams, 1999; Ollila and Larmas, 2007; Sayegh et al., 2005; Tsai et al., 2006) or between carbonated soft drinks and cavitated caries (Al-Malik et al., 2001; Mariri et al., 2003; Marshall et al., 2003; Marshall et al., 2005; Sohn et al., 2006). Only one study (Ohlund et al., 2007) reported no significant association between candy/sugar, soft drink and sweet product consumptions with cavitated caries. One study (Nunn et al., 2009) that assessed eating patterns and early childhood caries (ECC) reported that children with the best eating practices (uppermost tertile) were less likely to manifest severe ECC (AAPD definition). One study (Thitasomakul et al., 2009) found that when the consumption of soft drinks or sugary snacks started at 9 months of age there was a significant association with having new caries.

A small number of English language studies have reported the prevalence and/or number of non-cavitated lesions (initial enamel caries) in the primary dentition (Autio-Gold and Tomar, 2005; Bankel et al., 2006; Carvalho et al., 1998; Holbrook et al., 1995; Poulsen and Malling Pedersen, 2002; Ramos-Gomez et al., 2002; Warren et al., 2002). However, only four studies used non-cavitated caries as an outcome variable for analytical purposes. The first study (Ramos-Gomez et al., 2002) categorized children with only incipient caries as a separate category from ECC; ECC was categorized as including children with only cavitated caries or including children with both non-cavitated and cavitated caries. Descriptive analyses of the risk factors were presented; however, only the associations between cavitated caries and diet and feeding practices variables were assessed. Two studies (Bankel et al., 2006; Holbrook et al., 1995) reported separately the prevalence of children with initial caries and the prevalence of children with cavitated caries. However, only overall caries, combining non-cavitated caries with cavitated caries, was assessed for association with dietary factors. One study (Kolker et al., 2007) did not report the distribution of caries; however, the authors used the combination of non-cavitated and cavitated caries as an outcome variable. None of these four studies examined the relationships between dietary factors and non-cavitated caries individually or weighted non-cavitated caries relative to cavitated caries.

The main purpose of the present analysis was to examine risk factors for non-cavitated caries. The secondary purpose was to examine risk factors for cavitated caries. We compared dietary intakes, expressed as frequencies of consumption, from 36 to 60 months old 1) between children with non-cavitated caries only and caries-free children, 2) between children with cavitated caries only and caries-free children, and 3) between children with both non-cavitated and cavitated caries and caries-free children in the primary dentition.

Materials and Methods

Subjects in this study were part of the longitudinal Iowa Fluoride Study (IFS). Children and parents were recruited from eight Iowa hospitals from 1992 to 1995. The details of the study population and recruitment of IFS subjects have been published elsewhere (Levy et al., 1998). This study was approved by the Human Subjects Committee at the University of Iowa, and parents provided informed consent.

Three-day diet diaries were sent to parents when children reached each of the ages 1.5, 3, 6, 9 and 12 months and then every 4 or 6 months through 102 months of age (Marshall et al., 2005). The diaries aimed to collect the food and beverage consumptions for two weekdays and one weekend day. Specific, detailed information regarding consumption times, types of items and quantities were collected. For these analyses, dietary diaries at 36, 48 (if available) and 60 months were abstracted by a trained registered dietitian or diet technician. If the designated diary was missing, then substitution was made using the diary either 4 or 6 months preceding (or succeeding) the yearly diary.

Frequencies of food and beverage consumptions were abstracted from diaries. Foods and beverages consumed within a 30-minute interval counted as a single eating event. Multiple servings of the same beverage or food consumed within 30 minutes also were considered as one item (Marshall et al., 2005).

The eating events then were categorized into meals or snacks based on the time of consumption and nature of the foods. Eating multiple items from a broad variety of food groups, especially those with high protein and fat, was categorized as a meal, while eating of 1-2 items at a time was categorized as a snack. There were only three meals per day allowed, one during the morning, one at the middle of the day and one during evening hours. However, there were unlimited eating events allowed for snacks. Each beverage was categorized by type of beverage, while each food was categorized based on sugar and/or starch content (Marshall et al., 2005). Relevant categories for beverages included milk, 100% fruit juice, juice drink, powdered sugared beverage, regular (sugared) soda pop and water. Categories for foods were pre-sweetened cereals, unsweetened cereals, baked starch with sugar (e.g., cake, cookies), unprocessed starches (e.g., bread, baked potato), processed starches (e.g., potato chips, pretzels), sugar-based desserts (e.g., gelatin, ice cream, pudding), candy, and added sugar (e.g., table sugar, honey, brown sugar). More details related to the abstraction methods have been presented elsewhere (Marshall et al., 2005). The frequencies of consumptions from the diaries of each child were averaged at each age across the 3 days, and then results were averaged across the 2 (36 and 60 months) or 3 (36, 48 and 60 months) ages.

IFS questionnaires were sent to parents at the same time as the diaries. Average daily toothbrushing frequency and composite water fluoride levels were determined based on the data from these questionnaires. Composite water fluoride levels were determined from all sources of water (i.e., home and childcare bottled/filtered/tap water) at each time point for each individual child. Composite water fluoride levels from 36, 48 and 60 months (with substitutions similar to those of the diaries described previously) were averaged. Average daily toothbrushing frequency also was averaged from available 36- to 60-month data.

Socio-economic status (SES) was classified from baseline questionnaires into low, medium and high categories. Since this population was relatively high SES and had quite a number of parents who were graduate and professional students (with substantial education, but low income), the subjects were classified into three categories based on family income and mother's education at recruitment (1992-1995). Specifically, children from families with annual incomes of less than $30,000/year whose mother had 2 years or less in college were categorized as low SES. Children from families with annual incomes from $30,000-$49,999/year whose mothers had a graduate/professional degree, or from families with annual incomes of more than $50,000/year (regardless of the mother's educational level) were categorized as high SES. All others were placed in the middle SES category.

Dental examinations were conducted by trained and calibrated examiners when the children were approximately 5 years old. The examiners used a portable chair and halogen head-light (Warren et al., 2002). The teeth were dried and a DenLite® mirror (Welsh-Allyn Medical Products, Inc., Skaneatele Falls, NY) was used for improved lighting and transillumination. The examination was conducted primarily by visualization; however, any questionable caries condition was confirmed by explorer (Warren et al., 2002). The diagnostic criteria used in this study were with a d_1-d_3 system modified from the criteria of Pitts (Pitts and Fyffe, 1988; Pitts, 1997). The criteria used in this study did not differentiate cavitated enamel (d_2) and dentine lesions ($d_{3\text{-}4}$), so those lesions were categorized together as d_{2+}. The d_1 criteria for non-cavitated lesions were similar to d_1 criteria used by others (Warren et al., 2002).

There were 393 subjects who had dental examinations at about age 5 and dietary diaries at both 36 and 60 months (or their substitutions, see below), but not all of these subjects had a 48-month diary (or substitution). Children were categorized into discrete groups: 1) the d_1 group for children with non-cavitated caries only (no cavitated caries), 2) the $d_{2+}f$ group for children with only cavitated and/or filled caries (without non-cavitated caries), 3) the d_1 $d_{2+}f$ group for children with both cavitated and non-cavitated caries, and 4) the caries-free group for children with neither non-cavitated nor cavitated caries. Repeat exams by different examiners were conducted on 67 children. Inter-examiner reproducibility of classification of these 67 children into the 4 subgroups was assessed. The weighted Kappa statistic was 0.72, which is viewed as reflecting "good" reproducibility (Landis and Koch, 1977). In the multinomial logistic regression modeling, the 4 caries groups comprised the outcome categories, with the caries-free group serving as the baseline or reference category.

For univariable **multinomial** logistic regression analyses, each dietary variable (consumption frequency/day) or other related factor (age at primary tooth dental exam, gender, brushing frequency, SES and composite water fluoride level) was modeled separately (results not shown). The results from these models were used to screen for potential associated factors (p-value < 0.15).

For the multivariable **multinomial** logistic regression modeling, dietary variables (consumption frequency/week) and other non-dietary factors with p-values less than 0.15 from the univariable analyses were initially considered for inclusion. These variables were examined for collinearity. Among variables with moderate to high pair-wise correlations (Pearson coefficients > 0.3), the variable with a weaker relationship with the outcome was removed. (The strength of the relationship was assessed by the size of the overall p-value in the univariable model.) All two-way interactions of these variables were assessed in bivariable models, except for those based on a product of two continuous variables. Interaction terms with p-values less than 0.15 were considered in the multivariable modeling. A backward elimination procedure was performed to determine the model, where each step in the procedure was specified only after evaluating the fitted model resulting from the previous step and considering changes in the Akaike information criterion (AIC). To assess the functional propriety of the fitted model (i.e., the relationship between the set of conditional log odds and the quantitative explanatory variables), we used the Hosmer and Lemeshow goodness of fit tests. Hosmer and Lemeshow (2000) suggested a simple way of testing the lack of fit of multinomial logistic regression model by checking goodness-of-fit tests for each comparison pair.

Multivariable **binomial** logistic regression modeling comparing risk factors between children in the d_1 group and children in the caries-free group was conducted using steps similar to those described for the multivariable multinomial logistic modeling. Then, variables significant in the binomial model but not in the multinomial model were identified and included in developing the final multinomial logistic regression model.

All data were processed in SAS 9.12 (SAS Institute Inc., Cary, NC, USA) and analyzed using PROC LOGISTIC.

Results

There were 393 children included in the analyses. About 52% of subjects were girls and most families were of middle or high SES (38% and 40%, respectively). The mean age at the primary dentition exam was 5.1 (±0.3) years old. The mean number of d_1 surfaces among those in the d_1 group was 2.27 (±1.78), the mean number of $d_{2+}f$ surfaces among those in the $d_{2+}f$ group was 4.89 (±5.14), and the mean number of d_1 and $d_{2+}f$ surfaces among those in the $d_1d_{2+}f$ group were 2.80 (±2.82) and 5.69 (±7.77), respectively.

Table 1 summarizes descriptive analyses for the 4 groups: the caries-free group, the d_1 group, the $d_{2+}f$ group and the $d_1d_{2+}f$ group. The table presents the percentages of children who had consumed the specific food item at any of the 2-3 available time points, and the medians, 25th and 75th percentiles of food and beverage consumption frequency data separately at snacks and meals.

Means of age at dental examination, toothbrushing frequency and composite water fluoride level and distribution of SES level for the 4 groups are presented in Table 2. More caries-free children were in the high SES level.

The Hosmer and Lemeshow goodness-of-fit tests for all univariable models (results not shown) revealed no instances of lack of fit.

For the multivariable **multinomial** logistic regression analyses assessing factors associated with being in the 4 caries outcomes groups, 6 dietary variables (3 food variables, 3 beverage variables) and 3 non-dietary variables were examined for collinearity, resulting in the removal of one variable (regular pop consumption at meals). Eight variables were then considered in the initial multivariable model. Due to missing data (14 for SES, 2 for toothbrushing frequency), there were 377 children used in the analyses. The results for the model from the use of AIC results in conjunction with the backward elimination procedure are shown in Table 3. Consumption frequencies of regular soda pop consumption at snacks and sugar at snacks, toothbrushing frequency, age at primary dentition, and SES level were retained in the model.

For the multivariable **binomial** logistic regression analyses assessing factors associated with being in the d_1 group vs. caries-free group, 6 dietary variables (2 food variables, 4 beverage variables) and 2 non-dietary variables were examined for collinearity and none was removed (Table 4). Eight variables were then considered in the initial multivariable model. Due to missing data (11 for SES, 1 for toothbrushing frequency), there were 248 children used in the analyses. Consumption frequencies of milk at meals and pre-sweetened cereal at meals, toothbrushing frequency and SES level were significant in the model.

Consumption frequencies of milk at meals and pre-sweetened cereal at meals were two variables that were not represented in the multinomial model (Table 3), but were significantly associated with being in the d_1 group in the binomial model (Table 4). These two variables and 5 others variables (from Table 3) were used to form an overall multivariable multinomial logistic regression model comparing the 3 caries groups with the caries-free group (Table 5). AIC results for models in Tables 3 and 5 showed that both models characterize the data about equally well, with neither clearly superior based on balancing parsimony and goodness-of-fit. Since the two additional variables were significantly associated with non-cavitated caries, which is our main emphasis, we chose the model including these two variables as our final model (Table 5).

Greater consumption frequency of regular soda pop at snacks significantly increased the likelihood of being in the $d_1d_{2+}f$ group (relative to the reference category, the caries-free group). Added sugar consumption at snacks significantly increased the likelihood of being in the $d_{2+}f$ group and the $d_1d_{2+}f$ group. Lower consumption frequency of the milk at meals and greater consumption frequency of pre-sweetened cereal significantly increased the likelihood of being in the d_1 only group. Greater daily toothbrushing frequency significantly decreased the likelihood of being in the $d_1d_{2+}f$ group and nearly (p-value =0.06) significantly decreased the likelihood of being in the d_1 group. Older children were significantly more likely to be in the $d_1d_{2+}f$ group. Children from higher SES families generally had decreased likelihood of being in any of the 3 caries groups. However, these relationships were statistically significant for the d_{2+f} and d_1 groups, but not for the $d_1d_{2+}f$ group.

Discussion

In the present study, the results from univariable logistic regression analyses were used to screen for variables to include in the multivariable logistic regression analyses. Thus, the discussion of the present study focuses on the results of the final multivariable logistic regression model.

In this multivariable modeling, the data was analyzed using both multinomial logistic regression analyses for 4 categorical outcomes and binary logistic regression analyses comparing the d_1 only group and caries-free group. Both variable screening and initial model selection in the multinomial regression analyses were based on the overall p-values. These p-values provide a composite characterization of the effect of a variable on the conditional odds defined by comparing each caries group to the caries-free reference group. Milk and pre-sweetened cereal consumption frequencies at meals were the two variables that were not statistically significant in the multinomial logistic analysis, but were significant in the binomial logistic analysis, as these two variables have weaker associations with the cavitated caries groups.

The main purpose of these analyses was to examine risk factors for non-cavitated caries, but non-cavitated caries is one stage of the caries process. Thus, conducting binomial logistic regression analysis alone (d_1 only vs. caries-free) does not seem adequate, since it would exclude subjects with cavitated caries. However, our initial multinomial logistic regression analysis (Table 3) does not explicitly assess factors associated with non-cavitated caries. The additional binomial logistic regression analysis led to the inclusion of the two additional factors in the final multinomial model, both of which were statistically significant (Table 5).

The final multinomial analysis (Table 5) needs to be considered within the limitations of the analysis. This analysis is unique, for there have been no published articles that have analyzed the non-cavitated caries outcome as a separate category from cavitated caries in the same analysis. Moreover, this analysis had small sample sizes for the affected groups. Thus, the results should be interpreted with caution. More studies with larger and more diverse sample are needed that simultaneously analyze non-cavitated and cavitated caries.

There are no other studies that have assessed the association between SES and non-cavitated caries in the primary dentition. In a systematic review (Reisine and Psoter,

2001) of cavitated caries studies, the authors reported that the available data demonstrated a fairly consistent significant inverse relationship between caries prevalence and SES. However, for more recent studies, one study (Petti et al., 2000) found a significant association between lower SES and cavitated caries experience, but the others (Enjary et al., 2006; Ramos-Gomez et al., 2002) did not find such an association. Socioeconomic status might represent other health-related factors that are difficult to measure or could not be assessed and were not available for inclusion in the multivariable logistic regression models, but are possibly related to dental caries, such as effective oral home care and patterns of food choices. The present study found that higher SES levels were significantly associated with being less likely to be in the d_1 only group than in the caries-free group. Higher SES was also statistically significantly associated with lower chance of being in the $d_{2+}f$ group than in the caries-free group. Thus, SES is an important variable which should be considered along with other related factors in multivariable regression models assessing the associations between both cavitated and non-cavitated caries.

One study (Mascarenhas, 1998) has reported no significant association between increased toothbrushing frequency and enamel caries (D_1 and D_2). In our study, frequent toothbrushing was marginally associated (p-value = 0.06) with being in the d_1 group and was significantly associated (p-value =0.02) with being in the $d_1d_{2+}f$ group. However, toothbrushing frequency was not significantly associated with being in the $d_{2+}f$ group. These results suggest that frequent toothbrushing might be a significant factor only for the d_1 caries component in both the d_1 group and $d_1d_{2+}f$ group. The reasons for this are unclear, but at 36 months of age, 98.7% of the subjects were using a fluoride dentifrice with brushing, and that percentage increased to at least 99% thereafter. Hence, the toothbrushing variable really was a measure of frequency of topical fluoride exposure and mechanical cleaning. Since studies generally do not find plaque levels or oral hygiene status to be associated with caries outcome (Mattila et al., 2001; Tagliaferro et al., 2008), we assume that it was mostly the topical fluoride exposure that reduced caries initiation in the present analyses. However, the findings of the present study are different than the conclusion of Groeneveld (Groeneveld, 1985), who found that water fluoridation had only a small preventive effect on initiation of dental caries (but a larger effect on dentinal caries). More studies are needed to assess the association between non-cavitated caries (d_1 and D_1) and toothbrushing frequency.

The relationship between daily toothbrushing frequency and prevalence of cavitated caries has been investigated extensively in preschoolers (Al-Malik et al., 2001; Douglass et al., 2001; Gibson and Williams, 1999; Reisine et al., 1994; Rodrigues and Sheiham, 2000). Three of five studies (Gibson and Williams, 1999; Reisine et al., 1994; Rodrigues and Sheiham, 2000) found a significant association between less frequent toothbrushing and increased risk of cavitated dental caries. One study (Al-Malik et al., 2001) reported no association between toothbrushing frequency and dental caries, after adjusting for other variables in a multivariable logistic regression. Additionally, another study (Douglass et al., 2001) reported higher brushing frequency was associated with greater cavitated caries experience prevalence. Like the findings from the Douglass et al. (Douglass et al., 2001) and Al-Malik et al. (Al-Malik et al., 2001) studies, the present analyses do not support a preventive effect of greater toothbrushing frequency on cavitated caries. One possible reason for this finding is that children with cavitated caries in these studies, especially those with substantial proportions of filled lesions as in the present study, could have brushed their teeth more often because they received more oral hygiene instruction from dental personnel during the treatment and/or prevention visits than did caries-free children. This could explain why frequent toothbrushing did not decrease the chance of being in the caries-free group in these observational studies. It is also possible that some children who had better access to care were in the cavitated caries group as a result of over-treatment of dental caries. These children might receive a preventive effect of frequent toothbrushing, however, they would be classified in the cavitated caries group due to the overtreated lesions. Thus, research studies with populations with better access to care might not find associations as strong between frequent toothbrushing and having less cavitated caries.

Pre-sweetened breakfast cereals have been available in the United States for many years. Such cereals have been prepared by coating the cereal pieces with a slurry or solution of sweeteners and then drying the cereals in an oven or air current. There is an average of about 42g of total sugar in 100g of pre-sweetened cereals (Wheeler et al., 1996). Thus, an association between increased exposures to pre-sweetened cereals at meals and dental caries is highly plausible. There are no published studies that have examined the relationship between non-cavitated caries and pre-sweetened cereals. In the current study, pre-sweetened cereal consumption frequency at meals was significantly associated with the d_1 group in the multivariable multinomial logistic regression model.

One animal study (McDonald and Stookey, 1977) that examined the relationship between gross caries and pre-sweetened cereals in hamsters reported that pre-sweetened cereals were more cariogenic than non-pre-sweetened cereals. Another study (Gibson, 2000) examined the association between cavitated caries and pre-sweetened cereals in humans. The author analyzed data from the 1995 UK National Diet and Nutrition Survey of children aged 1.5 to 4.5 years, and reported no association between pre-sweetened cereal consumption frequency and cavitated caries prevalence. Pre-sweetened cereal consumption frequency at meals was not significantly associated with being in either the $d_{2+}f$ group or the $d_1d_{2+}f$ group in the present analyses.

Children with greater milk consumption frequency at meals were significantly more likely to be in the caries-free group than in the d_1 group in the multivariable logistic regression model. No other published study has assessed the relationship between milk consumptions and non-cavitated caries. For cavitated caries, one study which assessed the effect of milk consumption on cavitated caries experience of both primary and permanent teeth in Italian children age 6-11 years old (Petti et al., 1997) found that milk consumption was a protective factor only in the group of children with high sucrose consumption. They did not find this association in the groups of children with moderate and low sucrose consumptions.

There are several studies that have assessed the association between regular soda pop consumption frequency and cavitated caries experience. Al-Malik et al. (Al-Malik et al., 2001) and Sohn et al. (Sohn et al., 2006), as well as several earlier publications from the Iowa Fluoride study (Mariri et al., 2003; Marshall et al., 2003; Marshall et al., 2005), reported a strong positive association between regular soda pop consumption frequency and cavitated caries experience in preschool children. The present analyses found that greater regular soda pop consumption at snacks increased the likelihood of being in the $d_{2+}f$ group and significantly increased the likelihood of being in the $d_1d_{2+}f$ group compared with being caries-free. However, the present analyses did not find a significant association between being in the d_1 group and regular soda pop consumption frequency either at snacks or at meals. There are no published studies that have examined an association between regular soda pop consumption frequency and non-cavitated caries in preschool children.

The greater consumption frequencies of regular soda pop consumption and added sugar that were significantly associated with being in the $d_{2+}f$ and/or in the $d_1d_{2+}f$ groups were the consumption frequencies at snacks (not at meals). Children with high consumptions of either of these two variables at snacks were significantly more likely to be in the cavitated caries groups, which is the more advanced stage of dental caries. The greater consumption of pre-sweetened cereal and lower consumption of milk at meals, where they are consumed with some other foods and/or beverages, were significantly associated with being in the d_1 group. Consumption of high cariogenicity foods and beverages at meals is believed to decrease the cariogenicity of such consumption (Marshall et al., 2005). Children with d_1 only did not have significantly greater consumption of regular soda pop and added sugar at snacks (with high cariogenicity) than those caries-free children; however, these children had significantly greater consumption of pre-sweetened cereals at meals (with low cariogenicity) and lower milk consumption frequency at meals than those caries-free children.

Several studies (Al-Malik et al., 2001; Ferreira et al., 2007; Gibson and Williams, 1999; Milgrom et al., 2000; Sohn et al., 2006; Tsai et al., 2006; Watson et al., 1999) have found a significant association between age at dental examination and cavitated caries experience. The present analyses produced similar results. There is no other published study that has assessed the association between age and non-cavitated caries. Cavitated caries experience is an irreversible state that results in increased prevalence and number of affected surfaces over time. On the other hand, non-cavitated caries is a reversible stage between a sound surface and cavitated lesion. Thus, time might not be the primary significant factor, but the factors that change the dynamics of demineralization and remineralization of non-cavitated caries could be more important factors.

While several studies used questionnaires to collect information about specific food types (Ollila and Larmas, 2007; Ramos-Gomez et al., 2002; Sayegh et al., 2005; Tsai et al., 2006), several others collected dietary data using a frequency questionnaire that focused on a selected number of snacks and sugar-containing foods and beverages and the consumption frequency per time period (week or day) (Al-Malik et al., 2001; Bankel et al., 2006). This study (Mariri et al., 2003; Marshall et al., 2005), along with several other studies (Gibson and Williams, 1999; Ohlund et al., 2007; Sohn et al., 2006), had the advantage of using dietary records (3-5 days) or 24-hour recall interviews to collect more complete records of food and beverage consumption frequency and/or quantity and time. This increase in data collection items, combined with the data abstraction method, provided detailed data separately for consumption at meals and snacks.

Most of the published studies on this subject have assessed overall frequency of food and/or beverage consumption per day. For some food and beverage consumptions, the combined consumption at meals and snacks together may not show an association with caries, but separate analyses of only meal or snack consumptions may exhibit an association with dental caries (Marshall et al., 2005). The combining of these two types of consumption in other studies would tend to weaken the associations.

The idea of separately assessing risk factors for different stages of caries is not new. However, there are a limited number of studies, because most of the earlier studies used diagnostic criteria that did not separate enamel caries from dentin caries. There are two studies that have assessed the different risk (or protective) factors for enamel caries (D_1 and D_2) and dentine caries (D_3, D_4, filled and missing due to caries) (Groeneveld, 1985; Mascarenhas, 1998). The present study classified children into 4 discrete groups at the person-level; thus we could separately assess the risk factors for non-cavitated caries. However, we used a different stage as a cutoff, in which our criteria concentrated on cavitation status regardless of the depth of the lesions.

There are several limitations of the present study. First, the study group was predominantly white and mostly of middle and high SES, so generalization of the results to the general population is restricted. Second, no "activity level of caries" was measured or scored, thus the study cannot distinguish the association between diet and active vs. inactive non-cavitated caries. Third, this study did not use radiographic examination; therefore, the caries prevalence would tend to be underestimated due to undiagnosed proximal carious lesions. Fourth, only one examination of primary teeth was used in the analyses. This report did not investigate rates of progression or incidence of non-cavitated carious lesions.

The present study had a fixed sample size, which also is a limitation. Usually, studies that examine risk factors for dental caries manage type I error by setting the significance level at 0.05. However, the relationships between consumption frequencies of specific types of foods or beverages, and caries outcomes are complex and possibly subtle, since many different foods and beverages are ingested in combination and at varied times. Thus, studies of this type, including the present analyses, should also be concerned about the level of type II error, since lower type II error rates would allow more potential explanatory variables to be identified. Since the relationship between the type I and type II error rates is such that an increase in type I error probability means a reduction in type II error probability, using a strict level of significance in exploratory studies increases the likelihood of overlooking a potentially important risk factor. This is the first report that tried to separately examine the risk factors for non-cavitated and cavitated caries (in the same analysis). More and larger studies which have detailed dietary data and use examination criteria that could separately identify non-cavitated and cavitated/filled caries are needed.

Most studies of caries and risk factors, even those published this decade, have focused on cavitated caries as the only outcome variable. Selection of preventive measures to treat non-cavitated caries based on information from those cavitated caries studies may not be appropriate for prevention of non-cavitated caries. Thus, a better understanding of risk factors for non-cavitated caries could provide the framework for the development of preventive programs to substantially decrease the initiation of dental caries in children. In the future, more in-depth, longitudinal studies of caries progression over time should analyze the data using both cavitated and non-cavitated caries definitions.

In summary, the present study suggests frequent toothbrushing, milk consumption at meals and avoiding pre-sweetened cereal consumption at meals might reduce the risk of developing non-cavitated caries. Future research studies need to place more emphasis on investigation of risk factors for non-cavitated caries as part of assessing caries risk factors overall.

Table 1 Descriptive analysis of dietary variables (age 36-60 months old) by caries category (N=393)

Beverage (Occasions/Day)		% Children with Some Consumption* (N=393)	Median (25^{th}, 75^{th} Percentile)			
			Caries-free (N=257)	d_1 (N=41)	$d_{2+}f$ (N=46)	$d_1d_{2+}f$ (N=49)
Powdered Sugared Beverages	Snack	35%	0.0 (0.0, 0.1)	0.0 (0.0, 0.2)	0.0 (0.0, 0.3)	0.0 (0.0, 0.2)
	Meal	33%	0.0 (0.0, 0.1)	0.0 (0.0, 0.1)	0.0 (0.0, 0.2)	0.0 (0.0, 0.1)
Regular Soda Pop	Snack	48%	0.0 (0.0, 0.2)	0.0 (0.0, 0.2)	0.1 (0.0, 0.2)	0.1 (0.0, 0.3)
	Meal	71%	0.2 (0.0, 0.3)	0.2 (0.1, 0.4)	0.2 (0.1, 0.4)	0.2 (0.1, 0.4)
Juice Drinks	Snack	51%	0.0 (0.0, 0.2)	0.1 (0.0, 0.2)	0.1 (0.0, 0.2)	0.1 (0.0, 0.3)
	Meal	56%	0.1 (0.0, 0.2)	0.1 (0.0, 0.3)	0.1 (0.0, 0.2)	0.1 (0.0, 0.2)
100% Juice	Snack	81%	0.3 (0.1, 0.7)	0.3 (0.1, 0.6)	0.3 (0.1, 0.7)	0.3 (0.0, 0.6)
	Meal	84%	0.4 (0.1, 0.8)	0.3 (0.1, 0.7)	0.4 (0.1, 0.8)	0.3 (0.1, 0.8)
Milk	Snack	84%	0.3 (0.2, 0.7)	0.2 (0.1, 0.6)	0.3 (0.1, 0.8)	0.3 (0.1, 0.7)
	Meal	99.7%	1.8 (1.2, 2.2)	1.6 (1.0, 2.2)	1.7 (1.3, 2.1)	1.8 (0.9, 2.0)
Water	Snack	92%	0.8 (0.3, 1.3)	0.6 (0.2, 1.1)	0.6 (0.2, 1.1)	0.4 (0.2, 1.0)
	Meal	64%	0.1 (0.0, 0.3)	0.1 (0.0, 0.2)	0.1 (0.0, 0.3)	0.1 (0.0, 0.3)

Table 1 Continued

Food (Occasions/Day)		% Children with Some Consumption* (N=393)	Median (25th, 75th Percentile)			
			Caries-free (N=257)	d_1 (N=41)	$d_{2+}f$ (N=46)	$d_1d_{2+}f$ (N=49)
Pre-sweetened Cereals	Snack	29%	0.0 (0.0, 0.1)	0.0 (0.0, 0.1)	0.0 (0.0, 0.1)	0.0 (0.0, 0.0)
	Meal	85%	0.3 (0.1, 0.6)	0.3 (0.2, 0.7)	0.3 (0.2, 0.7)	0.3 (0.1, 0.4)
Unsweetened Cereals	Snack	20%	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)
	Meal	59%	0.1 (0.0, 0.2)	0.1 (0.0, 0.3)	0.1 (0.0, 0.2)	0.1 (0.0, 0.2)
Baked Starch with Sugar	Snack	98%	0.6 (0.4, 0.9)	0.7 (0.3, 0.9)	0.6 (0.3, 0.8)	0.6 (0.4, 1.0)
	Meal	86%	0.3 (0.2, 0.6)	0.2 (0.0, 0.4)	0.3 (0.1, 0.6)	0.3 (0.1, 0.4)
Unprocessed Starches	Snack	74%	0.1 (0.0, 0.3)	0.2 (0.1, 0.3)	0.2 (0.1, 0.3)	0.2 (0.1, 0.4)
	Meal	100%	2.6 (2.2, 2.8)	2.4 (2.0, 2.7)	2.3 (2.0, 2.8)	2.4 (2.2, 2.7)
Processed Starches	Snack	78%	0.2 (0.1, 0.4)	0.2 (0.1, 0.6)	0.2 (0.0, 0.3)	0.2 (0.0, 0.4)
	Meal	87%	0.3 (0.1, 0.6)	0.3 (0.2, 0.4)	0.2 (0.1, 0.4)	0.3 (0.2, 0.4)
Sugar-based Desserts	Snack	81%	0.2 (0.1, 0.3)	0.2 (0.1, 0.4)	0.3 (0.1, 0.4)	0.2 (0.0, 0.4)
	Meal	69%	0.2 (0.0, 0.3)	0.1 (0.0, 0.2)	0.1 (0.0, 0.2)	0.1 (0.0, 0.2)
Candy	Snack	72%	0.2 (0.0, 0.4)	0.1 (0.0, 0.3)	0.2 (0.0, 0.4)	0.2 (0.0, 0.6)
	Meal	36%	0.0 (0.0, 0.1)	0.0 (0.0, 0.1)	0.0 (0.0, 0.0)	0.0 (0.0, 0.1)
Added Sugar	Snack	33%	0.0 (0.0, 0.1)	0.0 (0.0, 0.1)	0.0 (0.0, 0.2)	0.1 (0.0, 0.2)
	Meal	80%	0.3 (0.1, 0.4)	0.2 (0.0, 0.4)	0.2 (0.0, 0.4)	0.2 (0.1, 0.4)

*Percentage of children with some consumption from diaries (among 2 or 3 annual time points per child).

Table 2 Descriptive analysis of other related factors (age 36-60 months old) by caries category (N=393)

Parameter	Caries-free (N=257)	d_1 (N=41)	$d_{2+}f$ (N=46)	$d_1d_{2+}f$ (N=49)
	Mean (SD)			
Toothbrushing Frequency (3 to 5 Years)	1.4 (0.5)	1.2 (0.5)	1.3 (0.5)	1.2 (0.4)
Average Composite Water Fluoride Level from Age 3 to 5 (ppm F)	0.8 (0.4)	0.9 (0.5)	0.8 (0.3)	0.8 (0.4)
Age	5.1 (0.3)	5.1 (0.3)	5.2 (0.4)	5.2 (0.5)
	Percent Distribution			
SES				
Low	17.3%	31.6%	31.1%	27.7%
Middle	34.5%	52.6%	42.2%	40.4%
High	48.2%	15.8%	26.7%	31.9%

Table 3 Multivariable multinomial logistic regression model to estimate the conditional odds of being in the d_1 only group (n=38) relative to the caries-free group (n=248), in the $d_{2+}f$ group (n=46) relative to the caries free group and the $d_1d_{2+}f$ group (n=45) relative to the caries free group

Parameter	d_1 only vs. Caries-free		$d_{2+}f$ vs. Caries free		$d_1d_{2+}f$ vs. Caries-free		Overall p-value
	Odds Ratio (95% CI)	p-value	Odds Ratio (95% CI)	p-value	Odds Ratio (95% CI)	p-value	
Regular Soda Pop at Snacks (Occasions/week)	1.01 (0.79, 1.41)	0.71	1.14 (0.89, 1.46)	0.31	1.35 (1.08, 1.67)	0.008	0.07
Added Sugar at Snacks (Occasions/week)	0.92 (0.56, 1.54)	0.75	1.59 (1.15, 2.19)	0.005	1.62 (1.18, 2.23)	0.003	0.005
Daily Toothbrushing Frequency (Time)	0.47 (0.22, 0.999)	0.0498	0.76 (0.39, 1.45)	0.40	0.40 (0.20, 0.82)	0.02	0.03
Age at Primary Dentition Exam (Year)	0.70 (0.23, 2.14)	0.54	2.00 (0.81, 4.92)	0.14	3.76 (1.55, 9.09)	0.004	0.02
SES Level (3 Levels)	0.46 (0.29, 0.74)	0.002	0.57 (0.37, 0.89)	0.02	0.71 (0.45, 1.12)	0.15	0.003

Note: The initial, full model additionally included frequencies of consumptions of powdered sugared beverages at snacks, sugar-based dessert at meals, candy at meals, and unprocessed starches at snacks. Added sugar at snacks includes table sugar, honey, and brown sugar.

Table 4 Multivariable binomial logistic regression model to estimate the odds of being in the d_1 only group relative to the caries-free group

Parameter	Odds Ratio (95% CI)	p-value
Milk at Meals (Occasions/Week)	0.91 (0.83, 0.98)	0.020
Pre-sweetened Cereal at Meals (Occasions/Week)	1.34 (1.10, 1.65)	0.005
Daily Toothbrushing Frequency (Time)	0.47 (0.22, 0.996)	0.049
SES Level (3 Levels)	0.43 (0.26, 0.70)	0.0007

Note: 1) The initial, full model additionally included frequencies of consumptions of powdered sugared beverages at snacks, regular soda pop at meals, juice drinks at meals, unprocessed starches at meals, age at primary tooth dental exam, gender, composite water fluoride level, and the interaction between powdered sugared beverage consumption frequency at snacks and SES level. These variables were removed in the backward elimination (p-value <0.05 to remain).

2) Included subjects are caries-free children (n=248) or those with non-cavitated caries experience (d_1) only (n=38); those with cavitated caries experience ($d_{2+}f$) are excluded.

Table 5 Multivariable multinomial logistic regression model including 2 variables* (from Table 4) to estimate the conditional odds of being in the d_1 only group (n=38) relative to the caries-free group (n=248) , in the $d_{2+}f$ group (n=46) relative to the caries free group and the $d_1d_{2+}f$ group (n=45) relative to the caries free group

Parameter	d_1 only vs. Caries-free		$d_{2+}f$ vs. Caries-free		$d_1d_{2+}f$ vs. Caries-free		Overall p-value
	Odds Ratio (95% CI)	p-value	Odds Ratio (95% CI)	p-value	Odds Ratio (95% CI)	p-value	
Regular Soda Pop at Snacks (Occasions/week)	1.01 (0.75, 1.36)	0.97	1.12 (0.87, 1.43)	0.40	1.32 (1.01, 1.64)	0.02	0.11
Added Sugar at Snacks (Occasions/week)	0.93 (0.56, 1.54)	0.77	1.58 (1.14, 2.18)	0.006	1.63 (1.18, 2.25)	0.003	0.006
Milk at Meals (Occasions/week)	0.91 (0.84, 0.99)	0.03	0.97 (0.90, 1.05)	0.42	0.96 (0.89, 1.04)	0.33	0.14
Pre-sweetened Cereal at Meals (Occasions/week)	1.30 (1.07, 1.59)	0.008	1.13 (0.95, 1.36)	0.18	1.00 (0.83, 1.22)	0.99	0.042
Daily Toothbrushing Frequency (Time)	0.47 (0.21, 1.03)	0.06	0.76 (0.40, 1.47)	0.42	0.41 (0.20, 0.83)	0.02	0.04
Age at Primary Dentition Exam (Year)	0.63 (0.20, 1.99)	0.44	1.91 (0.77, 4.73)	0.17	3.78 (1.56, 9.12)	0.004	0.02
SES Level (3 levels)	0.43 (0.26, 0.71)	0.0009	0.56 (0.36, 0.88)	0.02	0.72 (0.53, 1.14)	0.17	0.002

* These two variables that were significant in the binomial logistic regression model for d_1 vs. caries-free (Table 4), but did not meet the screening criterion (p-value <0.15) for the multinomial regression models.

CHAPTER V

MIXED DENTITION CAVITATED CARIES INCIDENCE AND DIETARY INTAKE FREQUENCIES

Abstract

Caries is a common disease in school-children even in higher socioeconomic communities. *Purpose:* We examined risk factors for children having new cavitated caries from age 5 to 9 years. *Methods:* Subjects were Iowa Fluoride Study cohort children (mostly white and relatively high socioeconomic status) with both primary and mixed dentition caries exams and 2^+ diet diaries from age 5 to 8 years (n=198). Using surface-specific transitions, combined counts of new cavitated caries ($d_{2\text{-}3}f$ and/or $D_{2\text{-}3}F$) from four primary second molars, eight permanent incisors and four permanent molars were determined. Food and beverage intake frequencies were abstracted. Other factors were assessed using periodic questionnaires. Logistic regression identified predictors of new cavitated caries. *Results:* Thirty-seven percent had new cavitated caries. The mean new cavitated caries count for all children was 1.17 surfaces (SD 2.28). In multivariable logistic regression, age 5 non-cavitated caries experience (OR=2.67, *P*=0.03), age 5 cavitated caries experience (OR=3.39, *P*=0.004), greater processed starch at snack frequency (OR=3.87, *P*=0.07), being older (OR=1.68, *P*= 0.04) and less frequent toothbrushing (*P*=0.001) were significantly associated (*P*<0.10) with having new cavitated caries. *Conclusion:* Results suggested that increased toothbrushing frequency and reduced consumption of processed starches as snacks may reduce caries incidence in younger school-aged children.

Introduction

Dental caries remains a major public health problem for U.S. children. The National Health and Nutrition Examination Survey (NHANES) 1999-2002 reported a national prevalence of 49% children with dental caries experience in the primary teeth and 20% of children with caries experience in the permanent teeth (Beltran-Aguilar et al., 2005) for 6- to 11-year-old children. Early school-age children are at a stage of transition from the primary dentition to the mixed dentition. Moreover, they also begin elementary school, which generally changes the beverage and food intake patterns.

Most studies have assessed risk factors for dental caries in the mixed dentition cross-sectionally. Since the dental caries process takes time to develop into clinically detectable lesions, risk factors for dental caries should be assessed by examining factors that occur before and during the time of clinical caries detection. However, there are a limited number of non-experimental studies that have assessed caries incidence/increment in the transition period from primary to mixed dentition.

Associations between previous cavitated caries and new cavitated caries in primary school-age children have been investigated (Leroy et al., 2005; Peretz et al., 2003; Skeie et al., 2004; Steiner et al., 1992; Tagliaferro et al., 2008; Vallejos-Sanchez et al., 2006; Vanobbergen et al., 2001; Zhang and van Palenstein Helderman, 2006). Consistently, findings show a strong and significant association between previous caries experience and new caries. Since previous dental caries experience is not a modifiable factor, studies should examine modifiable risk factors for dental caries, beyond previous caries experience. Few studies (Leroy et al., 2005; Mattila et al., 2001; Tagliaferro et al., 2008; Vanobbergen et al., 2001) have included modifiable factors, such as dietary intakes, fluoride usage and toothbrushing frequency. Two of these studies (Mattila et al., 2001; Vanobbergen et al., 2001) found significant associations between consumption of sweets/sweetened drinks and new cavitated caries, while the other two(Leroy et al., 2005; Tagliaferro et al., 2008) found no significant association between dietary consumption (sweets, sugar or snacks) and new cavitated caries.

The objective of the analyses described in this paper was to examine modifiable and non-modifiable risk factors for children having new cavitated caries from age 5 to 9 years. The frequencies of dietary intake of specific food and beverage categories and other caries-related factors were compared between children with new cavitated caries and those without new cavitated caries from the ages of 5 to 9 years.

Methods

The data used in this analysis are part of the ongoing, longitudinal Iowa Fluoride Study (IFS). Subjects were recruited immediately postpartum from 8 Iowa hospitals and followed thereafter by mail and periodic assessments/exams. The children were mostly white and relatively high SES(Levy et al., 2003). Details of the recruitment of IFS subjects have been published elsewhere (Levy et al., 1998). This study was approved by the Human Subjects Committee at the University of Iowa. Parents of the participating subjects provided informed consent and children provided assent for the exams.

Subjects

Subjects with both primary dentition (~age 5 years) and mixed dentition (~age 9 years) caries exams and at least two diet diaries including at least one from 5 to 6.5 years and one from 7 to 8.5 years (to ensure at least one diary before starting school and at least one after) were included in these analyses (n=198). Details of the caries exams and dietary information are presented below.

Dietary Information

The food and beverage intakes for two weekdays and one weekend day were recorded by parents using three-day diaries that were sent every 1.5 months to 6 months (from 1.5 months to 8.5 years of age)(Marshall et al., 2005). Detailed information of consumption times, types of items and quantities were collected. Annual dietary diaries (i.e., at 5, 6, 7 or 8 years) were abstracted by trained registered dietitians or diet technicians. If the 5-, 6-, 7- and/or 8-year diary was missing, then substitution was made using the diaries 6 months succeeding or preceding the yearly diary.

Food and beverage intake frequencies were estimated from the diaries (Marshall et al., 2005)and categorized so that consumption within a 30-minute interval counted as one eating event. More than one serving of the same beverage or food consumed within 30 minutes was considered one event. The eating events then were identified as occurring as meals or snacks based, on the time of consumption and nature of the food. Only three meals per day were allowed, one during the morning, one at the middle of the day and one during evening hours. However, unlimited eating events were allowed for snacks.

Foods and beverages were then classified into categories. Beverages were categorized by type of beverage and included milk, 100% juices, juice drinks, powder sugared beverages, regular (sugared) soda pop, diet soda pop, sports drinks, and water. Foods were categorized based on sugar and/or starch content and included sugar-based desserts (jelly, pudding, etc.), candy, sugar (added sugar, table sugar, etc), baked starch with sugar (cookies, cake, etc.), unsweetened cereals, pre-sweetened cereals, unprocessed starches (boiled potato, bread, rice, etc.), and processed starches (potato chips, etc.). The intake frequency of each food and beverage category was counted and averaged for each child across the 3 days for each annual diary (at 5, 6, 7 or 8 years). Results at age 5 and/or 6 years and results at age 7 and/or 8 were averaged separately, and these two means were subsequently averaged to form an overall measure for each child. The dietary variables were presented in order by percentage of children with some intake (age 5-8 years) at snacking occasions in each of the beverage, sugar-based and starch-based food groups.

Other Related Factors

Daily toothbrushing frequency and composite water fluoride intakes were determined based on the data from IFS questionnaires that were sent to parents at the same time as the diaries. Composite water fluoride levels were determined from all sources, including available public water documentation and an assay of multiple sources of water (i.e., home/school, bottled/filtered/tap water) at each time point for each individual child(Levy et al., 2001; Levy et al., 2003). Composite water fluoride levels and daily toothbrushing frequency(Franzman et al., 2004) from all available 5- to 8-year data for each child were averaged.

Three categories of socio-economic status (SES) were created based on family income and mother's education data from baseline questionnaires (completed at birth). The high SES category included children from families with incomes from $30,000-$49,999/year whose mothers had a graduate/professional degree, or from families with incomes of more than $50,000/year (regardless of the mother's educational level). Children from families with incomes less than $30,000/year whose mother had 2 years or less in college were categorized as low SES. All other children were placed in the middle SES category.

Dental Data

Children were examined for dental caries at age 5 (primary dentition) and age 9 (mixed dentition) by the same trained and calibrated examiners. The examinations were conducted using a portable chair, halogen headlight and a DenLite® mirror (Welch-Allyn Medical Product, Inc., Skaneatele Falls, NY) (Warren et al., 2002). The teeth were dried and examined primarily by visualization. However, any questionable lesions were confirmed by explorer (Warren et al., 2002). This study used the diagnostic criteria that were modified from the D_1-D_4 system of Pitts and colleagues (Pitts and Fyffe, 1988; Pitts, 1997). The examiners did not differentiate cavitated enamel (D_2/d_2) and dentine lesions ($D_{3\text{-}4}/d_{3\text{-}4}$), thus those lesions were categorized together as $D_{2\text{-}3}/d_{2\text{-}3}$.(Warren et al., 2002)

Combined count of new cavitated caries ($d_{2\text{-}3}f$ and/or $D_{2\text{-}3}F$) included cavitated lesions and restored lesions from four primary second molars, eight permanent incisors and four permanent first molars. The counts of new cavitated caries were determined using surface-specific transitions between the age 5 and age 9 exams. Having new cavitated caries was used as the dependent binary variable (none vs. one or more new lesions and/or restorations) in these analyses.

Data Analysis

Logistic regression analyses were used to explore the relationships between having or not having new cavitated caries from ages 5 to 9 and other variables: dietary intakes, gender, SES, age at mixed dentition exam, cavitated caries experience at age 5, non-cavitated caries experience at age 5, tooth brushing frequency and composite water fluoride level.

First, univariable logistic regression analyses were modeled separately for each dietary variable or other related factor. For dietary variables, only variables with $P<0.15$ from the univariable logistic regression were selected for the next steps. The pairwise correlations between all pairs of continuous variables of selected dietary variables and other variables were determined. For those pairs with moderate to high correlations (Pearson-coefficients >0.3), only the variable with the stronger association with caries incidence would be included in an initial model for multivariable logistic regression analysis. All two-way interactions were then assessed in bivariable models, except for those based on a product of two continuous variables. Dietary variables, other related variables and interaction terms with $P<0.15$ (and the corresponding main effects) were considered in the multivariable modeling and were used to create two separate models. The two models were initially based on:

1. dietary and other variables (toothbrushing frequency, composite water fluoride level, gender, age at mixed dentition exam and SES) excluding previous dental caries experience, and
2. all variables (including previous caries experience).

For each of these model formulations, a backward elimination procedure was performed to determine the final model. Variables that did not retain significance at *P*

<0.10 were omitted sequentially based on the associated p-value. All data were analyzed using PROC LOGISTIC in SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

There were 198 children (55% girls) who met the inclusion criteria of having both exams and a sufficient numbers of diet diaries. Mean (SD) ages at the primary dentition and mixed dentition exams were 5.1 (± 0.4) and 9.2 (± 0.7), respectively. Most were in the middle or high SES categories (35% and 42%, respectively). The prevalence of non-cavitated caries at the primary and at the mixed dentition exams were 19.2% and 38.9%, respectively. Cavitated caries prevalence at the primary and at the mixed dentition exams were 21.2% and 49.0%, respectively. Sixty-three percent of the children had no new cavitated caries (zero surfaces of new cavitated caries). Among the 37% of children with some new cavitated caries, more than half (20%) had only 1-2 surfaces of new cavitated caries, about one-fourth (9%) had 3-4 surfaces of new cavitated caries, and less than one-fourth (8%) had 5 or more surfaces of new cavitated caries. The overall mean new cavitated caries for all children was 1.17 surfaces (SD of 2.28, range from 0 to 15), with a mean only among those affected of 3.2 surfaces (SD=2.8, range from 1 to 15).

Descriptive analyses presenting medians, 25^{th} and 75^{th} percentiles of food and beverage intake frequencies at meals and at snacking occasions for individuals' intakes for each year (ages 5, 6, 7 and 8) and averaged from the 2 to 4 diaries (age 5 to 8) are presented in Table 6. This table also presents the percentages of children who had consumed from each specific food/beverage category at ages 5, 6, 7, 8 and at any of the 2-4 available time points (age 5 to 8). Based on these percentages, milk was the most common drink for this age group (99%). Other common beverages were 100% juice, regular soda pop and juice drink. About one-fourth of the children consumed diet soda pop. Sports drinks were not a common beverage for these children (≤10%).

Medians, means and standard deviations of other related factors at ages 5, 6, 7, 8 and averages of any available data from age 5 to 8 are presented in Table 7. On average, children brushed 1.5 times per day and water fluoride levels fell into the optimal range of 0.7-1.2 ppm. More than three-fourth of the children did not have each of non-cavitated and cavitated caries at the primary dentition exam (81% and 79%, respectively).

Table 8 summarizes the univariable logistic regression analyses assessing the associations between having new cavitated caries and the numbers of occasions for the dietary variables. These analyses were used to screen the dietary variables for inclusion in the multivariable logistic regression. Significant variables ($P < 0.15$) were regular soda pop intake at snack, unprocessed starches intake at snacking occasions and processed starches intake at snacking occasions.

The univariable logistic regression analyses of factors other than dietary variables are presented in Table 9. Low SES was significantly associated with having new cavitated caries. Greater toothbrushing frequency was significantly associated with not having new cavitated caries, while gender, age at mixed dentition exam, and composite water fluoride level were not significantly associated with having or not having new cavitated caries. Having non-cavitated caries experience at age 5 (OR=4.00, 95% CI 1.91, 8.39, P <0.001) and having cavitated caries experience at age 5 (OR=4.44, 95% CI 2.16, 9.12, P <0.001) were significantly associated with having new cavitated caries from age 5 to 9.

The multivariable logistic regression analyses are presented in Table 10. Three dietary variables were initially included: regular soda pop intake at snack, unprocessed starches intake at snacking occasions, and processed starches intake at snacking occasions. There was no pairwise collinearity concern with the 6 continuous variables (2 food variables, 1 beverage variable, toothbrushing frequency, age at mixed dentition exam, and composite water fluoride level).

Model 1 initially considered eight variables, including 3 dietary variables and 5 other variables (daily toothbrushing frequency, composite water fluoride level, gender, age at mixed dentition exam and SES), and two interaction terms (between gender and composite water fluoride level and between low SES and age at mixed dentition dental exam). Greater processed starches intake frequency at snacking occasions (OR = 1.50, *P* =0.04) and low SES (OR=2.27, *P*=0.04) were significantly associated with having new cavitated caries. Greater daily toothbrushing frequency was significantly associated with not having new cavitated caries (OR=0.32, *P*=0.0009). Higher composite water fluoride level was significantly associated with not having new cavitated caries in girls; however, it was not significantly associated with not having new cavitated caries in boys. The *P* -value for the Hosmer and Lemeshow goodness-of-fit test was 0.72, indicating an adequate fit of the final model.

Model 2 initially included ten variables (8 variables from Model 1, non-cavitated caries experience at age 5 (yes/no) and cavitated caries experience at age 5 (yes/no)), and two interaction terms (between gender and composite water fluoride level and between low SES and age at mixed dentition dental exam). Having non-cavitated caries experience at age 5 (OR=2.67, *P* =0.03), having cavitated caries experience at age 5 (OR=3.39, *P* =0.004), greater intake frequency of processed starches at snacking occasions (OR=3.87, *P*= 0.07) and being older (OR=1.68, *P*= 0.04) were significantly associated (at *P* <0.10) with having new cavitated caries from age 5 to 9. Greater daily toothbrushing frequency was significantly associated with not having new cavitated caries (OR=0.28, *P* =0.001). Higher composite water fluoride level was significantly associated with not having new cavitated caries in girls (OR=0.88, *P* =0.08); however, it was not significantly associated with cavitated caries incidence in boys. The *P*-value for the Hosmer and Lemeshow goodness-of-fit test was 0.93 indicating adequate fit of the final model.

Although the main analyses have focused on intake frequency data, additional analyses were conducted using quantity of intake data. These generally showed similar directions and strengths of association to the analyses using frequency data. For example, in additional univariable analyses, both greater regular soda pop intake frequency and quantity at snacking occasions tended to increase risk of having new cavitated caries ($p=0.0495$ and $p=0.12$, respectively). Both greater regular pop intake frequency and quantity at meals tended to increase risk of having new cavitated caries, but were not statistically significant ($p=0.37$ and $p=0.43$, respectively).

Although not our first emphasis, the relationship between SES and previous non-cavitated caries and the relationship between processed starches intake frequency at snacking occasions and previous cavitated caries experience were explored. Thirty-three percent of children of low SES families had previous non-cavitated caries experience, while only 15% of children of middle/high SES families had previous non-cavitated caries experience. Additionally, 11% of children who had reported no intake frequency of processed starches at snacking occasions had previous cavitated caries experience, while 24% of children with some intake of processed starches at snack had previous cavitated caries experience.

Discussion

This study sample was predominantly white, relatively high SES and low-moderate caries risk. However, more than one third had new caries in the 4-year period and nearly 10% had an average of more than one new surface per year. Even in a low-moderate-risk population, caries in school-age children is still a public health problem.

Infection by cariogenic bacteria and the demineralization process on tooth surfaces are part of the true dental caries disease processes, but non-cavitated caries and cavitated caries are signs of the chronic disease that can be more reliably assessed clinically. Routine assessments of dynamic changes in bacteria and/or tooth demineralization that can be reversed or remineralized in a short time are not feasible clinically. Thus, the present study assessed signs of dental caries (clinical exam) and used them in determining the outcome variable of new cavitated caries.

From the descriptive analyses of the dietary variables, more than three-fourths of these children consumed regular soda pop and about one-fourth consumed diet soda pop. Diet soda pop has been available in the United States for more than 50 years. However, the results reveal that many more children consumed regular soda pop than consumed diet soda pop. In addition, consumption of pre-sweetened cereal was more common among children than consumption of unsweetened cereals.

Several other studies found a strong association between previous caries experience of the primary dentition and new caries of the mixed dentition (Leroy et al., 2005; Peretz et al., 2003; Skeie et al., 2004; Steiner et al., 1992; Tagliaferro et al., 2008; Vallejos-Sanchez et al., 2006; Vanobbergen et al., 2001; Zhang and van Palenstein Helderman, 2006). Our findings strongly support this conclusion. While presence of caries experience is a predictor of future caries, it is not possible to intervene and change previous caries experience. Therefore, studies should assess other risk factors besides previous caries experience.

The present analyses did not use a traditional 5% significance level but instead used a 10% significance level. The univariable results showed that there was a strong relationship between caries incidence and previous caries experience and weaker relationships between caries incidence and certain other explanatory factors, such as SES and processed starches intake at snacking occasions. In the multivariable logistic regression analyses, when a 0.05 significance level was used in the backward elimination procedure, dietary variables were not retained in the final multivariable model. Because previous caries experience and the dental caries outcome is the same disease, it is possible that they are sharing the same risk factors. Thus, we explored further the relationship between these factors and previous non-cavitated and previous cavitated caries experience. The results indicate associations between SES and non-cavitated caries experience and between processed starches intake frequency at snacking occasions and cavitated caries experience. Because of these relationships, the simultaneous inclusion of previous caries experience in a multivariable model containing the other explanatory factors reduced the contribution of the other factors. Therefore, the dietary variables which had weaker relationships with caries incidence would have been dropped out from the model with the traditional p-value of 0.05. Thus, we decided to use a larger p-value to allow those variables with weaker relationships to be considered in the multivariable logistic regression. These approaches allow us to examine other related factors, such as dietary and fluoride-related factors, which are modifiable factors, in contrast with previous caries experience which is not modifiable.

A study by Leroy et al. (Leroy et al., 2005), which included previous caries experience and other related factors in the multivariable model, reported similar findings in which other related factors that were significantly associated with new caries in the univariable analyses were not retained in the final model. Tagliaferro et al. (Tagliaferro et al., 2008) discussed the findings in their study that oral hygiene habits variable did not reach statistical significance in the final model because of the strong association between previous caries experience and caries incidence.

To show how explanatory variables affected each other in the model, we developed two separate models. Model 1 initially included all potential variables other than previous caries experience. In the backward elimination procedure, the results of using a level of significance of 0.05 or 0.10 were identical. In the final model, three modifiable factors, daily toothbrushing frequency, composite water fluoride level and processed starches intake frequency at snacking occasions, were significantly associated with having new cavitated caries. SES was also significant in the final model.

Model 2, which initially included all potential variables including previous caries experience, showed that both previous caries experience variables (non-cavitated and cavitated), and daily toothbrushing frequency were significantly associated with new caries. Processed starch intake frequency at snacking occasions and composite water fluoride level are the two variables in the final model that had *P*-values between 0.05 and 0.10. These two variables would have been trimmed from the model with a 0.05 significance level. Including previous caries experience in the model strengthened the association between the age at mixed dentition exam variable and new caries, which was found statistically significant in the final model. Low SES consistently has been found to be related to caries outcome in published studies. In Model 2, SES was not significantly associated with caries incidence, probably because SES was highly correlated with age 5 caries experience (already in the model).

Processed starches are common snacks in developed countries. The association between caries incidence and processed starches is not surprising. However, no studies have found this association in early school-aged children. Possibly there are no other studies that have detailed information on dietary intake at this level. Also, processed starch intake frequency at snacking occasions might be counted with other snack intakes in other studies. Thus, the effect of processed starch exposures on new caries might be part of the association between a combined dietary variable such as frequency of snack intake and new cavitated caries. Developmentally, children in this age group still need to have snacks. Thus, the defined findings of which snacks would increase chances of having new caries can be very useful for making recommendations.

Daily toothbrushing frequency is a strong preventive factor in this group of children. This confirmed the finding of other studies (Leroy et al., 2005; Vanobbergen et al., 2001). However, some other studies (Mattila et al., 2001; Tagliaferro et al., 2008) assessing children’s oral hygiene did not find significant associations with caries incidence. In this study, more than 99% of children used fluoridated toothpaste. Thus, the daily toothbrushing frequency was the major factor concerning the effect of fluoride exposure from toothpaste. Daily toothbrushing frequency is the modifiable factor that had the strongest relationship with new caries.

In Model 2, there was a statistically significant interaction effect between gender and composite water fluoride level. Specifically, increased composite water fluoride level showed a significant protective effect in girls, but not in boys. Additional studies are needed to confirm and explain these findings.

Most other studies of caries incidence and diet collected dietary information at only one point (either at the beginning or the end of this period) and related it to the new caries. This study collected the dietary information longitudinally to evaluate the relationship between the dietary intake during the period between the two dental examinations and cavitated caries incidence which is a major strength of the present study. However, there were limitations to the study, including a relatively small sample size, a predominance of subjects with high SES, and a low to moderate disease level in the cohort. Thus, results should be interpreted in light of these limitations. This study did not assess cariogenic bacteria. Thus, the results of this study did not control for differences in the cariogenic bacteria among individuals. Additionally, no analyses considered duration of exposures to foods and beverages.

For practicality, future research should focus more on modifiable factors. Moreover, it should assess risk factors at a detailed level that could lead to practical preventive measures.

Conclusions

1. Processed starches intake at snacking occasions, toothbrushing and SES are variables associated with caries, consistent with other studies in pre-school and school-age children.
2. In school-age children, many of those associations with modifiable factors are absent from multivariable models which include previous caries

experience. Studies concerning risk factors for dental caries should be focused to include and emphasize modifiable factors.

3. Results suggested that toothbrushing with fluoridated toothpaste and dietary counseling could help decrease the risk of new cavitated caries in early, school-aged children.

Table 6 Descriptive analysis of dietary variables*

Variable		Median (25th, 75th Percentile)					Children with Some Intake (%)†				
		Age 5-8 (n=198)	Age 5 (n=171)	Age 6 (n=173)	Age 7 (n=164)	Age 8 (n=150)	Age 5-8 (n=198)	Age 5 (n=171)	Age 6 (n=173)	Age 7 (n=164)	Age 8 (n=150)
Beverage (Occasions)											
Milk	Snack	0.2 (0.1, 0.4)	0.3 (0, 0.7)	0.3 (0, 0.7)	0 (0, 0.3)	0 (0, 0.3)	80%	56%	55%	40%	37%
	Meal	1.6 (1.2, 1.8)	1.7 (1.0, 2.0)	1.5 (1.0, 2.0)	1.3 (1.0, 2.0)	1.7 (1.0, 2.0)	99%	94%	97%	96%	95%
100% Juice	Snack	0.1 (0, 0.3)	0 (0, 0.3)	0 (0, 0.3)	0 (0, 0)	0 (0, 0)	65%	47%	33%	23%	17%
	Meal	0.3 (0.1, 0.6)	0.3 (0, 0.7)	0.3 (0, 0.7)	0 (0, 0.7)	0 (0, 0.7)	80%	58%	52%	48%	41%
Regular Soda Pop	Snack	0.1 (0, 0.3)	0 (0, 0.3)	0 (0, 0.3)	0 (0, 0.3)	0 (0, 0.3)	65%	30%	34%	32%	30%
	Meal	0.3 (0.1, 0.4)	0.3 (0, 0.5)	0 (0, 0.3)	0.3 (0, 0.3)	0 (0, 0.5)	80%	55%	47%	57%	49%
Juice Drinks	Snack	0.1 (0, 0.2)	0 (0, 0.3)	0 (0, 0)	0 (0, 0.2)	0 (0, 0)	57%	26%	23%	25%	23%
	Meal	0.2 (0, 0.3)	0 (0, 0.3)	0 (0, 0.3)	0 (0, 0.3)	0 (0, 0.3)	69%	32%	36%	37%	36%
Powdered Sugared Beverages	Snack	0 (0, 0.1)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	41%	18%	15%	17%	12%
	Meal	0 (0, 0.1)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	39%	18%	17%	13%	12%
Sport Drinks	Snack	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	8%	2%	2%	1%	2%
	Meal	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	5%	4%	2%	2%	5%

Table 6 Continued

Variable		Median (25th, 75th Percentile)					Children with Some Intake (%)†				
		Age 5-8 (n=198)	Age 5 (n=171)	Age 6 (n=173)	Age 7 (n=164)	Age 8 (n=150)	Age 5-8 (n=198)	Age 5 (n=171)	Age 6 (n=173)	Age 7 (n=164)	Age 8 (n=150)
Diet Soda Pop	Snack	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	19%	6%	8%	8%	6%
	Meal	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	23%	5%	8%	9%	11%
Food (Occasions)											
Sugar-based Desserts	Snack	0.3 (0.1, 0.5)	0.3 (0, 0.5)	0.3 (0, 0.7)	0.3 (0, 0.7)	0 (0, 0.3)	86%	55%	61%	51%	48%
	Meal	0.2 (0.1, 0.3)	0 (0, 0.3)	0 (0, 0.3)	0 (0, 0.3)	0 (0, 0.3)	78%	42%	47%	39%	42%
Candy	Snack	0.3 (0.1, 0.5)	0 (0, 0.3)	0.3 (0, 0.7)	0.3 (0, 0.7)	0 (0, 0.3)	81%	46%	51%	55%	48%
	Meal	0.04 (0, 0.2)	0 (0, 0)	0 (0, 0)	0 (0, 0.3)	0 (0, 0)	50%	22%	23%	27%	19%
Sugar	Snack	0 (0, 0.2)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	46%	19%	21%	14%	15%
	Meal	0.3 (0.2, 0.6)	0.3 (0, 0.7)	0.3 (0, 0.7)	0.3 (0, 0.7)	0.3 (0, 0.7)	86%	58%	65%	60%	55%
Baked Starch with Sugar	Snack	0.5 (0.3, 0.8)	0.7 (0.3, 1.0)	0.7 (0.3, 1.0)	0.3 (0, 0.7)	0.4 (0, 0.7)	96%	80%	77%	69%	69%
	Meal	0.5 (0.3, 0.7)	0.3 (0, 0.7)	0.3 (0.3, 0.7)	0.6 (0.3, 0.8)	0.6 (0.3, 0.7)	95%	64%	75%	79%	77%
Processed Starches	Snack	0.3 (0.1, 0.4)	0 (0, 0.3)	0.3 (0, 0.3)	0 (0, 0.3)	0 (0, 0.3)	81%	45%	52%	43%	45%
	Meal	0.3 (0.2, 0.5)	0.3 (0, 0.7)	0.3 (0, 0.7)	0.3 (0, 0.7)	0.3 (0, 0.7)	95%	65%	69%	68%	64%

Table 6 Continued

Variable		Median (25th, 75th Percentile)					Children with Some Intake (%)†				
		Age 5-8 (n=198)	Age 5 (n=171)	Age 6 (n=173)	Age 7 (n=164)	Age 8 (n=150)	Age 5-8 (n=198)	Age 5 (n=171)	Age 6 (n=173)	Age 7 (n=164)	Age 8 (n=150)
Unprocessed Starches	Snack	0.2 (0.1, 0.3)	0 (0, 0.3)	0 (0, 0.3)	0 (0, 0.3)	0 (0, 0.3)	81%	44%	44%	39%	42%
	Meal	2.5 (2.3, 2.8)	2.3 (2.0, 3.0)	2.5 (2.0, 3.0)	2.7 (2.0, 3.0)	2.7 (2.3, 3.0)	100%	100%	99%	100%	99%
Pre-sweetened Cereals	Snack	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	24%	8%	9%	9%	7%
	Meal	0.3 (0.2, 0.5)	0.3 (0, 0.7)	0.3 (0, 0.7)	0.3 (0, 0.7)	0.3 (0, 0.5)	87%	56%	61%	59%	56%
Unsweetened Cereals	Snack	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	16%	6%	6%	5%	2%
	Meal	0.1 (0, 0.2)	0 (0, 0.3)	0 (0, 0.3)	0 (0, 0)	0 (0, 0.3)	55%	29%	22%	23%	23%

*To compute these measures, mean dietary intakes from ages 5 and/or 6 and ages 7 and/or 8 were calculated, and these two means were subsequently averaged.

†Percentage of children with some intake from diaries (among 2-4 annual time points per child).

Note: There were 22% additional abstracted dietary diary responses at ages 5 ½, 6 ½, 7 ½ and 8 ½ that are not included in the year-specific data, but are included as substitutes when age 5, 6, 7 and 8 were missing.

Table 7 Descriptive analysis of other related factors (age 5-8 years, n=198)

Variable	Median					Mean ± SD				
	Age 5-8 (n=198)	Age 5 (n=171)	Age 6 (n=173)	Age 7 (n=164)	Age 8 (n=150)	Age 5-8 (n=198)	Age 5 (n=171)	Age 6 (n=173)	Age 7 (n=164)	Age 8 (n=150)
Daily Toothbrushing Frequency	1.50	1.0	1.0	2.0	2.0	1.5±0.5	1.4±0.6	1.5±0.6	1.5±0.6	1.5±0.6
Composite Water Fluoride Level (ppm)	0.93	0.94	0.95	0.98	0.94	0.8±0.4	0.8±0.4	0.8±0.4	0.8±0.3	0.8±0.4
Non-cavitated Caries Experience		0					0.5±1.6			
Cavitated Caries Experience		0					1.2±4.3			

Table 8 Univariable logistic regression models using dietary variables to predict occurrence of new cavitated caries from age 5 to 9 (n=198)

Beverages/Foods (Occasions)		Odds Ratio (95% CI)	p-value
Milk	Snack	1.29 (0.64, 2.62)	0.48
	Meal	0.87 (0.52, 1.46)	0.60
100% Juice	Snack	0.90 (0.19, 4.40)	0.90
	Meal	0.79 (0.35, 1.78)	0.57
Regular Soda Pop	Snack	5.15 (1.003, 26.43)	0.0496*
	Meal	1.75 (0.52, 5.94)	0.37
Juice Drinks	Snack	1.09 (0.28, 4.19)	0.90
	Meal	0.53 (0.16, 1.78)	0.31
Powdered Sugared Beverages	Snack	2.04 (0.47, 8.89)	0.35
	Meal	0.44 (0.04, 5.14)	0.51
Diet Soda Pop	Snack	0.48 (0.03, 7.03)	0.60
	Meal	1.92 (0.05, 70.26)	0.72
Sugar-based Desserts	Snack	1.33 (0.48, 3.72)	0.59
	Meal	0.38 (0.08, 1.76)	0.22
Candy	Snack	0.77 (0.31, 1.95)	0.59
	Meal	0.54 (0.07, 4.14)	0.56
Sugar	Snack	4.42 (0.42, 47.10)	0.22
	Meal	0.50 (0.19, 1.34)	0.17
Baked Starch with Sugar	Snack	0.91 (0.38, 2.18)	0.83
	Meal	0.79 (0.31, 2.03)	0.63
Processed Starches	Snack	2.85 (0.83, 9.83)	0.10*
	Meal	0.79 (0.25, 2.49)	0.69
Unprocessed Starches	Snack	3.16 (0.76, 13.15)	0.12*
	Meal	1.16 (0.62, 2.18)	0.64
Pre-sweetened Cereals	Snack	0.43 (0.01, 23.54)	0.68
	Meal	1.72 (0.56, 5.28)	0.35

Note: Each line of this table represents a separate regression model.

* $P < 0.15$ used as a criterion to screen dietary variables for multivariable logistic regression

Table 9 Univariable logistic regression models using demographic variables and other variables to predict occurrence of new cavitated caries from age 5 to 9 (n=198)

Variable	Odds Ratio (95% CI)	p-value
Gender		
• Boys	1.01 (0.59, 1.89)	0.85
Age at Mixed Dentition Dental Exam	1.24 (0.83, 1.85)	0.29
Age Interval between 1st and 2nd Exam	1.20 (0.82, 1.74)	0.35
SES		
• Low	2.07 (1.04, 4.13)	**0.04***
• Middle/High	Ref.	
Daily Toothbrushing Frequency from (5 to 8 Years)	0.37 (0.20, 0.69)	**0.002***
Composite Water Fluoride Level from Age 5 to 8 (ppm)	0.91 (0.40, 2.04)	0.81
Non-cavitated Caries Experience at Age 5 (Yes)	4.00 (1.91, 8.39)	**<0.001***
Cavitated Caries Experience at Age 5 (Yes)	4.44 (2.16, 9.12)	**<0.001***

Note: Each line of this table represents a separate regression model.

* P <0.05

Table 10 Multivariable logistic regression models to predict occurrence of new cavitated caries from age 5 to 9 (n=198)

Models	Estimate	Odds Ratio (95% CI)	p-value
Model 1 (All Variables Excluding Previous Caries Experience* Initially Included)			
Processed Starches Intake at Snack (Occasion)	1.50	4.48 (1.14, 17.70)	0.04
Daily Toothbrushing Frequency 5-8 Years Old	-1.15	0.32 (0.16, 0.63)	<0.001
Low SES (Yes)	0.41	2.27 (1.08, 4.80)	0.04
Composite Water Fluoride Level (Age 5-8) (0.1 ppmF)			
• Girls	-0.16	0.86 (0.75, 0.98)	0.03
• Boys	0.06	1.06 (0.93, 1.20)	0.39
Model 2 (All Variables† Initially Included)			
Processed Starches Intake at Snack (Occasion)	1.35	3.87 (0.93, 16.16)	0.07
Daily Toothbrushing Frequency 5-8 Years Old	-1.29	0.28 (0.13, 0.59)	0.001
Non-cavitated Caries Experience at Age 5 (Yes)	0.49	2.67 (1.11, 6.42)	0.03
Cavitated Caries Experience at Age 5 (Yes)	0.61	3.39 (1.48, 7.78)	0.004
Age at Mixed Dentition Exam (Year)	0.52	1.68 (1.04, 2.73)	0.04
Composite Water Fluoride Level (Age 5-8) (0.1 ppmF)			
• Girls	-0.13	0.88 (0.76, 1.01)	0.08
• Boys	0.05	1.05(0.93, 1.20)	0.44

Note: The other variables initially considered for inclusion (not shown in the table) were removed in the backward elimination (*P* <0.10 to remain).

* All variables excluding caries experience: regular soda pop (occasion) at snacking occasions, processed starches (occasion) at snacking occasions, unprocessed starches (occasion) at snacking occasions, toothbrushing frequency, composite water fluoride level, gender, age at mixed dentition exam and SES

†All variables: regular soda pop (occasion) at snacking occasions, processed starches (occasion) at snacking occasions, unprocessed starches (occasion) at snacking occasions, toothbrushing frequency, composite water fluoride level, gender, age at mixed dentition exam, SES, previous non-cavitated caries and previous cavitated caries

CHAPTER VI

LONGITUDINAL ASSOCIATIONS BETWEEN CHILDREN'S DENTAL CARIES AND RISK FACTORS

Abstract

Dental caries is a common disease in children of all ages. It is desirable to know whether children with primary, mixed and permanent dentitions share caries risk factors. Objective: To assess the longitudinal associations between caries outcomes and modifiable risk factors. Methods: One hundred and fifty-six children in the Iowa Fluoride Study met inclusion criteria of three dental examinations (primary, mixed and permanent dentitions at 5, 9 and 13 years, respectively) and caries-related risk factor assessments preceding each examination. Surface-specific counts of new non-cavitated caries and cavitated caries at the primary, mixed and permanent dentition examinations were outcome variables. Explanatory variables were caries-related factors, including averaged beverage exposure frequencies, toothbrushing frequencies, and composite water fluoride levels collected from 3 to 5, 6 to 8, and 11 to 13 years, dentition category, socioeconomic status and gender. Generalized Linear Mixed Models (GLMMs) were used to explore the relationships between new non-cavitated or cavitated caries and caries-related variables. Results: Greater frequency of 100% juice exposure was significantly associated with fewer non-cavitated and cavitated caries surfaces. Greater toothbrushing frequency and high SES were significantly associated with fewer new non-cavitated caries. Children had significantly more cavitated caries surfaces at the mixed dentition examination than at the primary and permanent dentition examinations. Conclusions: There were common caries-related factors for more new non-cavitated caries across the three dentitions, including less frequency of 100% juice exposure, toothbrushing frequency and socioeconomic status. A common modifiable caries-related factor for new cavitated caries was frequency of 100% juice exposure.

Introduction

Dental caries is a multifactorial disease that occurs in the complex environment of the oral cavity. Fermentable carbohydrates are known substrates for acidogenesis by cariogenic bacteria. The associations between dental caries and dietary risk factors have been examined for several decades. Non-dietary caries-related factors including fluoride, plaque/cariogenic bacteria, oral hygiene care/toothbrushing, demographic characteristics and previous caries experience also have been studied. Studies assessing caries risk factors have reported different results, due in part to different designs, analysis methods, subjects' ages and definitions of risk factors and caries.

Among studies assessing the associations between dental caries and dietary exposures in children, most studies investigated associations between cavitated caries experience and specific types of foods and beverages. Some studies reported significant associations between cavitated caries and sugar sweetened drink or confectionary or candy consumption (Ollila and Larmas, 2007; Sayegh et al., 2005; Tsai et al., 2006). However, some other studies (Burt et al., 1988; Holbrook et al., 1995; Ismail et al., 2008; Levine et al., 2007) have not found significant associations between cavitated caries and candy, sugar consumptions or total non-milk extrinsic sugar (NMES) drinks.

Many studies (Holbrook et al., 1995; Teanpaisan et al., 2007) showed a positive correlation between dental caries and mutans streptococci levels. Other studies (Milgrom et al., 2000; Ramos-Gomez et al., 2002) reported no significant association between salivary mutans streptococci and caries experience. Studies (Leroy et al., 2005; Tagliaferro et al., 2008) that examined associations between previous caries experience and new caries outcomes consistently reported strong, significant positive findings. However, previous caries experience is not modifiable. Moreover, to obtain information on previous caries experience, expensive dental examinations that require dental professional personnel are needed.

Additional studies are necessary to better understand these complicated relationships. Moreover, changes in people's environment, culture and lifestyle have driven the need for new studies, some of which have been conducted recently using more current caries diagnostic criteria. Additionally, the interactions among risk factors and preventive factors require more complicated analyses which can lead to a better understanding of the associations between these caries-related factors and caries outcomes.

There are quite a number of published studies assessing caries risk factors using cross-sectional designs (Chung et al., 1977; Milgrom et al., 2000; Ramos-Gomez et al., 2002; Sayegh et al., 2005) and some using prospective observational designs (Burt et al., 1988; Ollila and Larmas, 2007; Tagliaferro et al., 2008), but relatively few using a longitudinal design (Thitasomakul et al., 2009; Warren et al., 2006). However, none of those which collected data longitudinally have published findings using a repeated measures analytical approach.

Longitudinal study designs allow us to assess the changes in dental outcomes over time, including possibly through the primary, mixed and permanent dentitions. Moreover, longitudinal studies provide data that can be analyzed using a repeated measures (correlated data) framework which reduces unobserved ("not able to be assessed or not in the study") individual differences in the study. This analytical approach usually allows statistical inference to be made with fewer subjects, which increases the statistical power of studies with similar numbers of subjects in long-term follow-up longitudinal designs compared with cross-sectional designs.

We used longitudinal data from the Iowa Fluoride Study to assess caries risk factors separately for non-cavitated caries and cavitated caries in children across the primary, mixed and permanent dentitions. These analyses assessed the longitudinal associations between dental caries outcomes and modifiable risk factors, including beverage exposure frequencies, toothbrushing, and fluoride exposures, after controlling for demographic characteristics and proportion of concurrent new non-cavitated and cavitated carious lesions to surfaces at risk for caries.

Methods

The Iowa Fluoride Study recruited mothers and newborns from 8 Iowa hospital postpartum units from 1992-1995 and has been collecting fluoride, dietary and other related information associated with dental fluorosis and caries since children were 1.5 months old. This study was approved by the Human Subjects Review Committee of the University of Iowa initially and at least annually thereafter. Parental informed consent and subjects' assent were obtained separately for each dental exam. Dietary diaries were sent every 1.5 to 6 months until age 8.5 years, and separate questionnaires assessing dietary, toothbrushing and fluoride were sent until age 13 years. Dental examinations were conducted at approximately ages 5, 9 and 13. Cohort children (n=156) who had all 3 dental examinations of the primary, mixed and permanent dentition and had at least 2 abstracted dietary diaries during the age 3-5 and 6-8 years periods and at least 2 questionnaires during the age 11-13 years period were included in this analysis.

Dietary Information

Diaries

Three-day dietary diaries were sent to parents every 1.5 to 6 months from age 1.5 months to 8.5 years to assess the food and beverage consumptions for two weekdays and one weekend day. Specific information regarding types of items consumed by children and details of consumption were collected. The yearly dietary diaries at ages 3, 4 and 5 years (for the age 3 to 5 period) and 6, 7 and 8 years (for the age 6 to 8 period) were abstracted by a trained registered dietitian or diet technician to provide dietary exposure frequency (occasions per day) of specific food and beverage categories. If the particular yearly diary was missing, then substitution was made using the diary either 4 or 6 months preceding (or succeeding, if necessary) the yearly diary. More detailed information concerning the dietary diary data collection was published previously (Marshall et al., 2005).

Since, response rates to the dietary diaries decreased over time, the protocol was changed after age 8.5 years, at which time the dietary diaries were discontinued and replaced with a detailed beverage consumption questionnaire.

Questionnaires

The new detailed questionnaires have been sent to parents every 6 months since age 9 years to assess the exposure frequencies (occasions per day) of specific beverage categories, as well as other information. The questionnaires returned at ages 11, 11.5, 12, 12.5 and 13 years were used for the age 11- to 13-year period.

Beverage Exposure Variables

For these analyses, the beverage exposure data were compiled for the following six categories: milk, water by itself, reconstituted sugared beverage from powder (i.e. powdered beverages), regular (sugared) soda pop, 100% juice, and juice drinks by type of beverage and fermentable carbohydrate type. Uncommon beverage categories (i.e., those for which less than 25% of children had any exposure during each period) were excluded from the analysis, including sport drinks and diet soda pop. Within each exposure period (3-5, 6-8 and 11-13 years), the frequencies of beverage consumption were averaged for each child. Each subject had 3 sets of explanatory data, one for each exposure period. The 3 sets of explanatory variables were not averaged but were used as concurrent variables for each of the dental outcomes (3-5 year exposures for exam 1, 6-8 year for exam 2 and 11-13 year for exam 3). These period-specific exposure frequencies then were categorized into low, middle and high frequency levels using the approximate 25th and 75th percentiles of exposure frequency for each beverage category and exposure period. However, due to the prevalence of tied values, children were not always divided exactly into 25%, 50% and 25% for low, middle and high levels of exposure, respectively, as targeted.

Fluoride and SES

Toothbrushing frequency data were collected using the same questions on both the detailed questionnaires from age 6 weeks to 8.5 years and the questionnaires used since age 9 years (Franzman et al., 2004). Composite water fluoride levels were determined at all time points as weighted averages of the main sources of water (i.e., home/school, bottled/filtered/tap water) at each time point (Levy et al., 2001). For those using public water without filtration, water fluoride information was obtained from the Iowa State Health Department. For individual water sources or public water with filtration, water samples were collected and fluoride levels determined by individual fluoride assay with a fluoride-ion specific electrode (Levy et al., 2001). All available toothbrushing frequency and composite water fluoride level data for each period (3-5, 6-8 and 11-13 years) were averaged separately for each child. Questionnaire reliability was assessed on a sample by telephone about 7-10 days after receipt of the questionnaires for selected questions, including tooth brushing frequency. Reliability results (weighted kappa) for toothbrushing frequency were 0.75, 0.81 and 0.73 for ages 3-5, 6-8 and 11-13 years, respectively.

Socioeconomic status (SES) was classified into low, middle and high based on family income and mother's education level information that was collected at recruitment (Hamasha et al., 2006).

Dental Caries Information

Dental caries examinations were conducted by trained examiners at about age 5 (primary dentition), age 9 (mixed dentition) and age 13 (permanent dentition) using non-cavitated/cavitated criteria (Warren et al., 2006) modified from Pitt's criteria (Pitts, 1997). The inter-examiner reliability results at the surface-level using weighted kappas to assess consistency of classification (sound, d_1/D_1 (non-cavitated caries), or $d_{2+}f/D_{2+}F$ (cavitated/filled caries)) for the primary, mixed and permanent dentitions were 0.81, 0.82 and 0.58, respectively. The outcome variables were calculated using surface specific transitions based on the changes of caries status of specific tooth surfaces for each individual child between two consecutive examinations (person-level caries).

For the primary dentition, counts of lesions of non-cavitated and cavitated caries/filled at the age 5 examination were used as new non-cavitated and new cavitated caries, respectively.

For the mixed and permanent dentitions, transitions of diagnosis from sound to non-cavitated lesions were counted as new non-cavitated caries and from sound/non-cavitated caries lesions to cavitated caries or filled lesions were counted as new cavitated caries. Surface-specific transitions of caries status (sound sound, d_1/D_1, or $d_{2+}f/D_{2+}F$) from the primary to mixed dentition exams were counted as new caries for the mixed dentition exam outcomes (Figure 1). Transition of caries status on specific surfaces from the mixed to permanent dentition exams were counted as new caries for the permanent dentition exam outcomes.

The counts of new non-cavitated and cavitated caries served as the dependent variables. All surfaces present at the primary dentition exam were counted as surfaces at risk for both non-cavitated and cavitated caries for primary dentition outcomes. For later exams, surfaces that were sound at the previous exam plus newly-erupted surfaces were counted as surfaces at risk for <u>non-cavitated</u> caries. Surfaces that were sound or d_1 at the previous exam and newly-erupted surfaces were counted as surfaces at risk for <u>cavitated</u> caries. The counts of surfaces at risk served as an offset variable. The offset variable, representing the number of surfaces at risk for non-cavitated and cavitated caries, was featured in all models. The proportion of <u>concurrent non-cavitated</u> caries surfaces to surfaces at risk was a control variable in the multivariable regression models for <u>cavitated</u>

caries outcome, while the proportion of concurrent cavitated caries surfaces to surfaces at risk was a control variable for non-cavitated caries outcome.

Analytical Methods

The nature of longitudinal data results in correlated data. This analysis used a repeated measures modeling framework that accounts for correlated outcomes from the same individual. The explanatory variables from ages 3 to 5 years, 6 to 8 years and 11 to 13 years were considered for associations with new cavitated and non-cavitated caries recorded at the primary dentition, mixed dentition and permanent dentition exams (Figure 1).

Descriptive statistics of dental caries outcomes, beverage exposure variables and other caries-related factors for each individual period were assessed. Generalized linear mixed models (GLMMs) based on the negative binomial distribution were used to explore separately the relationships between new non-cavitated or cavitated caries and caries-related variables: frequency of beverage exposures, tooth brushing frequency, composite water fluoride level, SES, gender, and types of dentition. The log link function was used, and accordingly, the offset variable (representing counts of surfaces at risk) was log transformed. Random effects were included in the GLMM to account for the correlation among the longitudinal outcomes for each subject. The random effects were assumed to be independent between subjects and to have a constant variance. The resulting subject-specific GLMM was fit using PROC GLIMMIX, which employs a pseudo-likelihood fitting technique.

First, univariable regression models were developed separately for each beverage and other caries-related variable. Only variables with p-values <0.15 from the univariable regressions were considered further for inclusion in the multivariable model. The pairwise correlations between pairs of continuous variables of selected beverage variables and other related factors were examined. For the moderate- to high-correlation pairs

(Pearson-coefficients >0.3), the plan was to retain only the variable exhibiting the stronger association with caries in the multivariable regression analysis. However, there were no occurrences of such correlations of pairs of variables. All two-way interactions among these considered beverage variables and other caries-related factors were then assessed, except for those based on a product of two continuous variables. The considered variables and interaction terms with p-values <0.15 (and the corresponding main effects) were included in the formulation of the initial multivariable model. A backward elimination procedure was performed to determine the final model, where each step in the procedure was specified only after evaluating the fitted model resulting from the previous step. Dentition type was a control variable (covariate), and was retained in the model regardless of the p-value to account for different ages and dental and oral environments at each examination. For other variables, the variable that had the largest p-value was removed from the model sequentially (except the corresponding main effects for interactions that were retained in the model). This step was repeated until all the variables remaining in the model were significant at the 0.05 level. All analyses were conducted using SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

One hundred and fifty-six children (45% female) were included in these analyses. Most children were of high or middle SES (37.8% and 34.4%, respectively). Mean ages at the primary, mixed and permanent dentition examinations were 5.15 ±0.38, 9.17 ±0.76 and 13.20 ±0.35 years, respectively.

For the primary dentition exam, fewer children had any non-cavitated caries (21.15%) when compared with the mixed (39.10%) and permanent (35.90%) dentitions exams (Table 11). The mean number of new non-cavitated caries surfaces among all children for the primary dentition exam (0.56) was lower than those for the mixed (0.99) and permanent (0.87) dentition exams. However, the mean number of new non-cavitated caries surfaces among only those children with any new non-cavitated lesions for the primary dentition (2.67) was slightly greater than those for the mixed (2.54) and permanent (2.41) dentitions. For the mixed dentition exam, about 54% of the children had new cavitated caries vs. 26-28% for the primary and permanent exams. However, among those with new cavitated caries, the means were about 5 new cavitated caries surfaces to about age 5 years, 5.5 surfaces from about age 5 to age 9 years and 2.4 surfaces from about age 9 to 13 years. Table 12 also presents means of surfaces at risk for both non-cavitated and cavitated caries by examinations.

Table 12 presents ranges of mean exposure frequency of each specific beverage category for low, middle and high levels at each of the 3 individual periods. Descriptive analysis results of mean daily toothbrushing frequency and composite water fluoride level for each period are presented in Table 13.

Results from the separate univariable regression analyses (Table 14) assessing the associations between number of surfaces with new non-cavitated caries and cavitated caries and the levels of the beverage exposure frequencies were used to screen the dietary variables for inclusion in the multivariable regression. The only statistical significant dietary variable (p-value < 0.15) for non-cavitated caries was frequency level of 100% juice exposure, while two variables were significant for cavitated caries: frequency level of 100% juice and powdered beverages exposures.

Table 15 summarizes the separate univariable regression analyses assessing the associations between new non-cavitated and new cavitated caries and factors other than dietary variables. Lower SES level and a higher proportion of concurrent new cavitated caries surfaces to surfaces at risk were significantly associated with more new non-cavitated caries. Greater toothbrushing frequency was significantly associated with fewer new non-cavitated caries, while gender, type of dentition and composite water fluoride level were not significantly associated with new non-cavitated caries. Greater proportion of concurrent new non-cavitated caries surfaces to surfaces at risk was significantly associated with more new cavitated caries surfaces in the univariable regression. Significantly more cavitated caries were found in the primary and mixed dentition than in the permanent dentition. Gender, SES, toothbrushing frequency and composite water fluoride level were not significantly associated with new cavitated caries.

Variables initially included in the multivariable regression model for new non-cavitated caries (Table 16) were: 1) 100% juice exposure frequency, 2) toothbrushing frequency, 3) proportion of concurrent new cavitated caries surfaces to surfaces at risk, 4) SES, 5) type of dentition, 6) the interaction term between toothbrushing frequency and type of dentition and 7) the interaction term between proportion of concurrent new cavitated caries surfaces to surfaces at risk and SES. Four variables were significantly associated (p-value < 0.05) with new non-cavitated caries surfaces in the final model: greater 100% juice exposure frequency, greater toothbrushing frequency and high SES were associated with fewer new non-cavitated surfaces, while a higher proportion of concurrent new cavitated caries surfaces to surfaces at risk was significantly associated with more new non-cavitated caries surfaces. Dentition type was not significantly associated with new non-cavitated caries.

Table 17 presents results of the multivariable regression model for new cavitated caries. Variables initially included were: 1) 100% juice exposure frequency, 2) powdered beverage exposure frequency, 3) toothbrushing frequency, 4) proportion of concurrent new non-cavitated caries surfaces to surfaces at risk, 5) type of dentition, and 6) the interaction term between powdered beverage and proportion of concurrent new non-cavitated caries to surfaces at risk. Three variables were significant in the final model (p-value < 0.05): 100% juice exposure frequency, proportion of concurrent new non-cavitated caries surfaces to surfaces at risk, and dentition type. A higher proportion of concurrent new non-cavitated caries surfaces to surfaces at risk was significantly associated with greater number of surfaces of new cavitated caries. Greater frequency of 100% juice exposure was significantly associated with fewer new cavitated caries surfaces. Children at the mixed dentition exam had a significantly greater number of cavitated caries surfaces than they did at the primary and permanent dentition exams.

Discussion

Most previously published studies of dental caries and caries-related factors were cross-sectional and examined the relationships between risk factors and caries outcomes at one time (concurrently) or at one subsequent time (prospective studies). The present study examined repeated caries outcomes longitudinally, with the risk factors at the ages of 3-5, 6-8 and 11-13 years associated with caries outcomes at the primary, mixed and permanent dentition examinations.

The strengths of this study include the prospective longitudinal design that collected detailed data on fluoride, dietary, other caries-related factors since recently after birth and dental caries outcomes of the primary, mixed and permanent dentitions. Detailed dietary information collected systematically by dietary diaries and detailed questionnaires allowed us to classify beverage exposures into meaningful categories for assessing caries risk factors.

Another strength of this study is that it used a mixed models approach that considers both fixed effects (explanatory variables) and random effects (individual differences). Thus, the individual differences among subjects were accounted for in our modeling framework. Moreover, the repeated outcomes for individual subjects allowed us to better control for individual differences. This improves the statistical power of inferential analyses. Also, this present analysis allowed us to assess the effect of risk factors jointly for the same children over three different age groups. Thus, the results indicated the overall, general effect of risk factors on dental caries across the different age periods and dentitions.

The present study also has several limitations, including subjects being almost all white children, mostly middle to high SES, and with low to moderate caries levels.

Moreover, there were only 156 children who met the criteria due to the extensive follow-up period and these statistical analyses required all three dental exams plus risk factor data for all three periods prior to the exams.

New non-cavitated caries and new cavitated caries were more common for the mixed dentition than for the primary and permanent dentitions. A longer duration of presence in the mouth and risk for caries for some teeth (about 7 years for primary molars) might be a reason for higher new cavitated caries for the mixed dentition. The difference was more pronounced for cavitated caries than for non-cavitated caries, probably because cavitated caries is an irreversible stage of the disease, in contrast with non-cavitated caries where there are chances to reverse to sound surfaces over the longer period. Among children with any cavitated caries, the lowest mean number of surfaces with new cavitated caries was in the permanent dentition. The shorter time since eruption and greater chances for preventive measures (sealants, professional and/or home fluoride use, etc.) might be reasons for lower caries for the permanent dentition.

Tooth brushing frequencies generally were higher for older children. Composite water fluoride levels (determined from home/school, bottled/filtered/tap water) were slightly lower on average for the older children. Increased usage of bottled water, which generally had lower fluoride levels, could have affected the composite water fluoride level.

The associations between dental caries and beverage exposure frequencies were not consistent dose-response relationships for most of the exposure categories. Therefore, the beverage variables were treated as categorical (not ordinal) variables in both univariable and multivariable regression analyses.

Frequency of 100% juice exposure was significantly related in the multivariable models to both new non-cavitated and cavitated caries. The negative association indicated lower caries in children with higher 100% juice exposure. There are two published studies that reported significant associations between greater 100% juice consumption and less caries. The first study (Kolker et al., 2007) reported a significant association between greater 100% juice consumption (excluding orange juice) and fewer surfaces with caries experience (cross-sectionally at about age 4 years). The other (Clancy et al., 1977) reported a significant negative correlation between fruit juice consumption and caries increment in adolescents. Also, in the previous IFS published study on age 5 caries experience and cavitated caries, there was a general tendency for greater daily 100% juice exposure at age 3, 4, 5 and for ages 1-5 combined to be associated with lower caries, but results were not statistically significant (Marshall et al., 2005). However, greater 100% juice exposure has not otherwise been demonstrated in human incidence studies or laboratory experiments to be a protective factor by itself. Certainly, the sugar content in 100% juice is a source of fermentable carbohydrate. There are studies that report higher risk of dental caries for higher 100% juice exposures with bottle-feeding (Du et al., 2007; Lewis et al., 2002).

There are several possible explanations for this preventive association with caries. First, 100% juice exposure might not be a protective substance by itself; however, low 100% juice exposure might relate to an exposure pattern of other cariogenic beverages. Moreover, the 100% juice exposure might indicate more healthy beverage or food choices for the individual overall. Second, some 100% juices have been demonstrated to have anti-bacterial effects from the non-nutrients (phytochemicals) that might potentially protect against or inhibit the caries process in the oral cavity (Cranberry juice for prophylaxis of urinary tract infections--conclusions from clinical experience and research, 2008). Third, different compositions of sugars in 100% juice (fructose/sucrose/glucose) compared to other sugared beverages might explain the lower cariogenicity of 100% juice compared with other sugar-added beverages, especially those with high fructose corn syrup (Marshall et al., 2003). There is a need for both laboratory and epidemiological studies to better explain the association between 100% juice exposure and dental caries.

Greater consumption of 100% juice was associated with less caries. However, juice has been associated with malnutrition in children. Substitution of 100% juice consumption for other sugared beverages might be recommended. However, the total amount of 100% juice should be less than 4-6 ounces daily according to American Academy of Pediatrics recommendations. Beyond that juice intake level, the beverages should be primarily milk, water and other non-caloric beverages.

The directions and strength of associations between dental caries outcomes and non-dietary caries-related factors were similar in both the univariable and multivariable regression models. This indicated that the dietary variables did not modify these associations. Thus, the results from both univariable and multivariable models can be interpreted and discussed together.

Greater toothbrushing frequency was consistently associated with fewer new non-cavitated caries surfaces and tended to be associated with fewer new cavitated caries surfaces. In this study, almost all children (>99%) used fluoridated toothpaste. Moreover, inconsistency of the associations between oral hygiene and/or plaque index and caries has been reported in other published studies (Chung et al., 1977; Sayegh et al., 2005). For the present study, we suggested that the protective effect of frequent tooth brushing is mainly from exposure to fluoride in toothpaste.

Studies assessing associations between SES and cavitated caries outcomes found mixed results. Most of the earlier studies (Beck et al., 1992; Brown et al., 1990; Evans et al., 1993) found strong relationships between SES and caries, but several more recent ones (Burt et al., 1988; Petti et al., 2000) did not. This probably is due in part to different definitions of caries outcomes, SES, etc., but also may be due to the fluoridated dentifrice and water ubiquity, great variety of dietary exposures and complexities of self-care behaviors. High SES was associated with smaller numbers of new non-cavitated caries in the present analysis. SES was categorized based on mother’s education and family income. Greater number of new non-cavitated caries in lower SES children could be explained by lower frequency of seeing the dentist for both prevention and treatment in this group of subjects and generally less favorable preventive behaviors. For cavitated caries, the present study found a trend of fewer new cavitated caries for those of high SES, however, the association was not statistically significant. Higher SES children usually have better access to care that might result in better prevention, fluoride exposures and counseling, which collectively can result in lower caries. However, they also had greater chance to receive restorative treatment, which could have increased the likelihood of getting over-treatment of non-cavitated caries (which increased number of new cavitated caries surfaces). These might be reasons for the non-significant association between cavitated caries and SES in the present study.

Previous caries experience consistently has been found to be associated with increased risk of having caries. As discussed in other studies, strong associations between previous caries and new caries outcomes generally reduced the contribution of other weaker explanatory variables (Leroy et al., 2005; Tagliaferro et al., 2008). Excluding previous caries experience from multivariable regression models allowed us to better explore the relationships of other modifiable variables which generally have weaker associations with caries outcomes.

This study is the first of its type to look at children's caries risk factors in this way using repeated measures analysis with the primary, mixed and permanent dentitions. Such studies are needed because we might find common caries-related factors that could be used to develop general preventive measures in children. There are many studies that look at caries risk factors at specific ages. In theory, repeated measures analysis has advantages over the single outcome study. However, we cannot conclude that the repeated measures analysis is a better way to understand strategies for caries prevention. We need more such studies which have multiple waves of dental outcomes to determine whether this approach is well suited for assessing caries-related factors and which factors are more appropriate for this approach (for example, water fluoride levels and socioeconomic status might be associated with caries outcomes for all age groups similarly, but dietary factors might not).

The present analyses concerning non-cavitated and cavitated caries and risk factors across the 3 caries examinations (primary, mixed and permanent dentitions) can be summarized as follows;

1. Greater frequency of 100% juice exposures were related to both less new non-cavitated and cavitated caries.
2. Frequency of other beverage exposures were not found to be significantly associated with increased risk for either type of caries outcome. However, they may be more important for individual time periods.
3. Lower SES was significantly associated with greater non-cavitated caries counts.
4. Greater toothbrushing frequency (almost all with fluoride toothpaste) was more strongly associated with new non-cavitated caries counts than with new cavitated caries counts.

Table 11 Means (±SD) of caries outcomes and surfaces at risk at each of the three examinations

Parameter	Primary Dentition Examination	Mixed Dentition Examination	Permanent Dentition Examination
% with New Non-cavitated Caries	21.15%	39.10%	35.90%
New Non-cavitated Caries (Surfaces)*			
• All children	0.56 ±1.65	0.99 ±1.94	0.87 ±1.77
• Among children with new cavitated caries	2.67 ±2.73	2.54 ±2.40	2.41 ±2.25
% with New Cavitated Caries	26.28%	53.85%	27.56%
New Cavitated Caries (Surfaces)*			
• All children	1.30 ±3.42	2.97 ±4.45	0.67 ±1.42
• Among children with new cavitated caries	4.95 ±5.17	5.52 ±4.77	2.42 ±1.76
Surfaces at Risk for Non-cavitated Caries**	86.81 ±3.78	110.73 ±5.26	123.01 ±11.83
Surfaces at Risk for Cavitated Caries***	86.81 ±3.78	111.22 ±5.27	123.53 ±11.70

*New counts determined though age 5 for primary dentition exam outcomes, from primary to mixed dentition for mixed dentition exam outcomes and from mixed to permanent dentition for permanent dental exam outcomes.

**Sound at previous exam.

***Sound or non-cavitated caries at previous exam.

Table 12 Categories of daily frequencies of beverage exposures at each of the three periods

Variable	Age 3 to 5		Age 6 to 8		Age 11 to 13	
	Exposure Frequency (Occasions/Day)*	%	Exposure Frequency (Occasions/Day)*	%	Exposure Frequency (Occasions/Day)*	%
Powdered Beverages						
• Low	0	48.1	0	47.4	0	26.3
• Middle	0.01-0.33	25.6	0.01-0.22	21.8	0.01-0.29	47.4
• High	>0.33	26.3	>0.22	30.8	>0.29	26.3
Regular Soda Pop						
• Low	<0.11	17.3	<0.14	25.6	<0.14	18.0
• Middle	0.11-0.56	60.9	0.14-0.67	58.3	0.14-0.50	56.4
• High	>0.56	21.8	>0.67	16.0	>0.50	25.6
Juice Drinks						
• Low	0	29.5	<0.11	21.2	0	19.2
• Middle	0.01-0.44	38.5	0.11-0.5	55.8	0.01-0.36	59.6
• High	>0.44	32.1	>0.5	23.1	>0.36	21.2
100% Juice						
• Low	<0.44	25.0	<0.11	20.5	<0.03	22.4
• Middle	0.44-1.33	50.6	0.11-1.11	69.9	0.03-0.57	50.0
• High	>1.33	24.4	>1.11	9.6	>0.57	27.6
Milk						
• Low	<1.67	27.6	<1.33	18.6	<1.03	26.3
• Middle	1.67-2.78	48.7	1.33-2.33	53.2	1.03-2.43	59.6
• High	>2.78	23.7	>2.33	28.2	>2.43	14.1
Water by Itself						
• Low	<0.44	20.5	<0.5	23.7	<1.03	17.3
• Middle	0.44-1.56	55.8	0.5-1.89	52.6	1.03-2.43	57.1
• High	>1.56	23.7	>1.89	23.7	>2.43	25.6

*Averages of exposure frequency of 2-5 time points during age period.

Table 13 Descriptive analysis (Mean (± SD)) of other caries-related factors at each of the 3 periods

Variable*	Age 3 to 5	Age 6 to 8	Age 11 to 13
Toothbrushing Frequency	1.33 (±0.54)	1.50 (±0.51)	1.48 (±0.52)
Composite Water Fluoride (ppm)	0.82 (±0.37)	0.80 (±0.33)	0.74 (±0.31)

*Averages from all responses during age periods.

Table 14 Univariable negative binomial regression models* assessing the associations between new non-cavitated and cavitated caries and dietary variables (N=156)

Variable	New Non-cavitated Caries**		New Cavitated Caries***	
	Estimate	p-value	Estimate	p-value
Powdered Beverages (Overall p-value)	(0.72)		(0.003)	
• Low	Ref.	Ref.	Ref.	Ref.
• Middle	-0.11	0.61	-0.64	0.005
• High	0.07	0.74	0.15	0.52
Regular Soda Pop (Overall p-value)	(0.55)		(0.25)	
• Low	Ref.	Ref.	Ref.	Ref.
• Middle	-0.25	0.28	0.40	0.12
• High	-0.14	0.62	0.19	0.54
Juice Drinks (Overall p-value)	(0.72)		(0.72)	
• Low	Ref.	Ref.	Ref.	Ref.
• Middle	-0.17	0.46	0.15	0.54
• High	-0.18	0.48	0.22	0.42
100% Juice (Overall p-value)	(0.003)		(<0.0001)	
• Low	Ref.	Ref.	Ref.	Ref.
• Middle	-0.31	0.15	0.21	0.35
• High	-1.03	0.0007	-1.12	0.0005
Milk (Overall p-value)	(0.72)		(0.33)	
• Low	Ref.	Ref.	Ref.	Ref.
• Middle	-0.06	0.79	0.05	0.84
• High	-0.22	0.44	0.36	0.21
Water by Itself (Overall p-value)	(0.65)		(0.53)	
• Low	Ref.	Ref.	Ref.	Ref.
• Middle	0.08	0.72	-0.03	0.91
• High	-0.12	0.66	0.23	0.43

*Each row for the non-cavitated (second column) and for cavitated caries (fourth column) was from a separate regression model.

**Transitions are from sound to non-cavitated lesions.

*** Transitions are from sound/non-cavitated caries lesions to cavitated caries or filled lesions.

Table 15 Univariable negative binomial regression models* assessing the associations between new non-cavitated and cavitated caries and other related variables (N=156)

Variable	New Non-cavitated Caries**		New Cavitated Caries***	
	Est.[§]	p-value	Est.[§]	p-value
Daily Toothbrushing Frequency (Occasions)	-0.40	0.03	-0.36	0.08
Composite Water Fluoride (ppm)	-0.28	0.34	-0.18	0.57
Proportion of Concurrent New Non-cavitated Caries Lesions to Surfaces at Risk (10% changes)	-	-	0.48	0.0002
Proportion of Concurrent New Cavitated Caries Lesions to Surfaces at Risk (10% changes)	0.23	0.0004	-	-
SES				
• Low	0 (Ref.)		0 (Ref.)	
• Middle	0.07	0.80	0.03	0.92
• High	-0.61	0.03	-0.29	0.35
Gender				
• Males	0.11	0.60	-0.18	0.49
Dentition				
• Primary	0 (Ref.)		0 (Ref.)	
• Mixed	0.37	0.08	0.83	<0.0001
• Permanent	0.16	0.46	-0.69	0.002

*Each row for the non-cavitated (second column) and for cavitated caries (fourth column) was from a separate regression model.

**Transitions are from sound to non-cavitated lesions.

** *Transitions are from sound/non-cavitated caries lesions to cavitated caries or filled lesions.

[§] β estimates

Table 16 Multivariable negative binomial regression model assessing the associations between new non-cavitated caries and related factors (N=151)

Variable	New Non-cavitated Caries* (d_1s/D_1S)	
	Est.[§]	p-value
100% Juice Exposure Level		(0.041)
• Low	0 (Ref.)	-
• Middle	-0.47	0.042
• High	-0.69	0.02
Tooth Brushing Frequency	-0.40	0.044
Proportion of Concurrent New Cavitated Caries Lesions to Surfaces at Risk (10% changes)	0.74	0.004
SES		
• Low/middle	0 (Ref.)	-
• High	-0.55	0.02
Dentition		(0.17)
• Primary Dentition	0 (Ref.)	-
• Mixed Dentition	0.43	0.08
• Permanent Dentition	0.37	0.13

*Transitions are from sound to non-cavitated lesions.

[§] β estimates

Note: $p<0.05$ to be retained, except dentition type, a control variable.

Table 17 Multivariable negative binomial regression model assessing the associations between new cavitated caries and related factors (N=151)

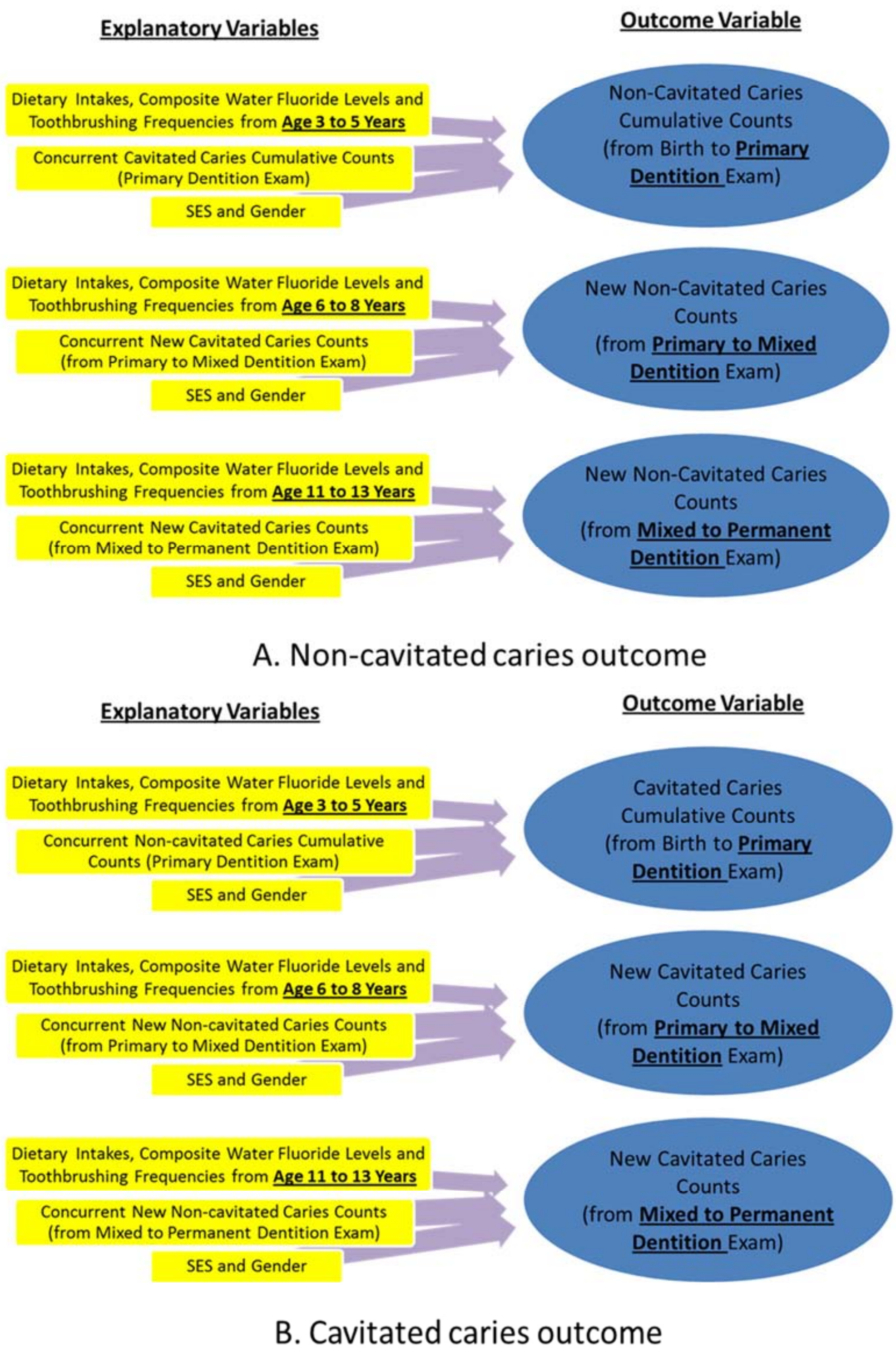
Variable	New Cavitated Caries* ($d_{2+}fs/D_{2+}FS$)	
	Est.[§]	p-value
100% Juice Exposure Level		(0.02)
• Low	0 (Ref.)	-
• Middle	0.07	0.76
• High	-0.65	0.03
Proportion of Concurrent New Non-cavitated Caries Lesions to Surfaces at Risk	1.26	0.007
Dentition		(<0.0001)
• Primary Dentition	0 (Ref.)	-
• Mixed Dentition	0.79	<0.0001
• Permanent Dentition	-0.67	0.003

* Transitions are from sound/non-cavitated caries lesions to cavitated caries or filled lesions.

[§] β estimates

Note: $p<0.05$ to be retained, except dentition type, a control variable.

Figure 1 Diagram showing the associations between explanatory variables and outcome variables for each individual period (A. for non-cavitated caries outcome and B. for cavitated caries outcome)



CHAPTER VII
GENERAL DISCUSSION AND CONCLUSIONS

Discussion

Introduction

This chapter will present a review of key findings, important aspects of the Iowa Fluoride Study (IFS), study limitations, power vs. statistical significance level, complex relationships between risk factors and caries, modifiable factors, previous caries experience, dietary variables, non-cavitated and cavitated caries, regression analysis vs. other approaches, extra-analyses, cross-sectional study vs. prospective study, analysis of repeated measurements, skewed outcome variables, future research directions, summary and clinical implications and conclusions. Specific results from this research are used to illustrate the implications of the findings in the context of existing literature.

Key Findings

A summary of types of dental outcome variables, dietary variables and statistical approaches for each of the three manuscripts is presented in Table 18 (see next page).

In the first manuscript (primary dentition caries experience) presented in Chapter IV, children were classified based on their age 5 person-level caries experience into 4 caries groups: the caries-free group (reference group), the d_1 group, the $d_{2+}f$ group, and the $d_1d_{2+}f$ group. Multinomial regression analyses were conducted to compare the dietary consumption frequencies (from ages 3 to 5 years) of the children in the 4 caries groups after adjusting for other caries-related factors. Lower consumption frequency of milk at meals and greater consumption frequency of pre-sweetened cereal at meals were significantly associated with a greater likelihood of being in the d_1 group. Greater consumption frequency of regular soda pop at snacks was significantly associated with being in the $d_1d_{2+}f$ group. Greater consumption frequency of food containing added sugars at snacks was significantly associated with being in the $d_{2+}f$ and the $d_1d_{2+}f$ groups.

Table 18 Key information for the 3 manuscripts

Manuscript	Dental Outcome	Dietary Variables	Statistical approach
Age 5 Life-long Caries Experience (Chapter IV)	Categorical Variable • Caries-free group • d_1 group • $d_{2+}f$ group • $d_1d_{2+}f$ group	Continuous Variables	Multinomial Regression
Age 5-9 New Cavitated Caries Occurrence (Chapter V)	Dichotomous Variable • Any • None	Continuous Variables	Logistic Regression
Ages 5, 5-9 and 9-13 Counts of New Non-cavitated and Counts of New Cavitated Caries (Chapter VI)	Discrete Variable	Ordinal Variables	General Linear Mixed Model (Negative Binomial)

In the second manuscript (mixed dentition new cavitated caries) presented in Chapter V, new cavitated caries on each of 16 teeth (4 second primary molars, 4 first permanent molars, and 8 permanent incisors) from the primary to mixed dentition exams was determined. Logistic regression analysis was used to predict occurrence of new cavitated caries using dietary factors after adjusting for caries-related non-dietary factors. Greater processed starch consumption frequency at snacks was significantly associated with having new cavitated caries ($p = 0.04$ for the model excluding previous caries experience and $p = 0.07$ for the model with previous caries experience).

In the third manuscript (longitudinal caries outcomes) presented in Chapter VI, surface-specific counts of new non-cavitated caries and cavitated caries at each of the primary, mixed and permanent dentition examinations were determined and used as outcome variables. Negative binomial regression using the Generalized Linear Mixed Models procedure was conducted to assess separately the associations of 1) new non-cavitated caries and 2) new cavitated caries with caries-related dietary factors, after adjusting for caries-related non-dietary factors. Greater consumption frequency of 100% juice was significantly associated with both fewer new non-cavitated and fewer new cavitated caries surfaces.

Iowa Fluoride Study

The Iowa Fluoride Study was launched originally to assess the associations between childhood fluoride intake and dental fluorosis. This longitudinal study has collected data from study participants since birth. Three-day dietary diaries and questionnaires were developed specifically for the study and were used initially to quantify fluoride intake. The secondary research purpose was to examine risk factors for dental caries. Later on, when the cohort children were in their adolescence, the focus on caries risk factors became the main purpose for the study. The detailed dietary intake information is one of the study's strengths. There are only a limited number of dental studies that have collected such detailed dietary information. Additionally, this study has followed the children for more than 15 years. Repeated measurements of explanatory variables, including diet, fluoride and oral health-related behavior, as well as dental outcomes, have been collected.

Limitations

The IFS has several limitations. The children participating in the study were almost all white children, mostly middle to high SES relative to national standards, and mostly with low to moderate caries levels. Therefore, extrapolation of the study's results is not appropriate to high disease populations and minorities. However, the study's results could be used generally for the majority of the U.S. population with moderate and lower caries rates, for which there are a very limited number of published studies.

This study collected caries-related data longitudinally – more than 30 times during a 13-year-period. There were a limited number of children with complete data. In these analyses, simple averages for a time period (correlated with different numbers of responses within the period and at different ages were used. Modeling area-under-the-curve (AUC) or other approaches might refine/smooth the data and result in different findings. Additionally, caries-related data (diet, toothbrushing, and fluoride exposures) were self-reported (parent-reported).

This study did not distinguish “active” and “inactive lesions”; therefore, “no activity level” of caries was reported. Also, this study did not use radiographic examination; thus, the caries prevalence would be expected to be underestimated due to undiagnosed proximal carious lesions.

Power vs. Statistical Significance Level

The present study followed a cohort of children for more than 15 years; thus there was a fixed sample size. In general, studies usually are more concerned with type I error than type II error. Researchers usually set the significance level at p=0.05 to control type I error. For this fixed sample size, a reduction in the level of one type of error results in an increase in the level of the other type of error. The nature of the associations between consumption frequencies of foods and beverages and caries outcomes is complex and subtle. There are many different categories of foods and beverages and many products in each category that are ingested at various times and in different combinations. Thus, studies of this type should also be concerned about the level of type II error. More potential explanatory variables can be identified when studies use lower type II error rates. Therefore, we chose to relax the significance level in order to reduce the type II error rate in these exploratory studies and studies that examine the relationships between several dietary variables and caries outcomes.

In the analyses in Chapter V, we used the 0.10 significance level instead of 0.05 in the model with previous caries experience to allow additional modifiable variables to be identified. Using this relaxed significance level of 0.10, we identified processed starches consumption frequency at snacks and composite water fluoride level, whereas had we used the strict 0.05 level, we would have excluded them.

Complex Relationships between Risk Factors and Caries

Several factors are known to be associated with dental caries. However, the interrelations among these factors increase the complexity of the relationships between these factors and dental caries. A factor that is found to be associated with significantly increased risk of dental caries in one population might not be a problem or might be a problem of substantially different magnitude in other populations, depending on the presence of other risk or protective factors in the same population. Also, different studies include different variables assessed in different ways, further complicating our ability to obtain consistent results.

Modifiable Risk Factors

Explanatory variables, including SES, age at dental examination, toothbrushing frequency and some categories of food/beverage consumption frequencies, were associated with dental caries outcomes in the present analyses. Toothbrushing frequency and food/beverage consumption frequencies are modifiable variables, but SES and age at dental examination are not. The modifiable factors could be used to develop guidelines and recommendations for caries prevention. Results obtained from studies such as this that have assessed the associations between specific food/beverage categories and caries are more appropriate for use in developing guidelines than are results from studies that use only total amount of sugar consumption per day or scores of a specific index as an explanatory variable. One study (Nunn 2009) assessed the association between a Healthy Eating Index (HEI) and dental caries. The HEI was assessed by an evaluation of 10 components, including 5 components regarding how a person's diet conforms to the pyramid guide and 5 other components concerning total fat and saturated fat, total food energy intake, total cholesterol, total sodium intakes and variety of dietary intake. There are studies (Karjalainen et al., 2001; Rodrigues and Sheiham, 2000) that assessed the association between total sugar consumption (g) and dental caries. Karjalainen et al. (Karjalainen et al., 2001) reported a significant association between higher daily sucrose consumption (g) and enamel and dentin caries in 6-year-old children. Another study (Rodrigues and Sheiham, 2000) found children having sugar more than 36.3 g/day were 2.99 times as likely to have high caries increment (3 or more surfaces) between age 3 and 4 years old. The results from these studies are more difficult to translate into clinical guidelines.

Previous Caries Experience

Including previous caries variable(s) in the regression model could affect the associations between caries-related factors and dental caries outcomes, since previous caries experience and the dental caries outcome are different aspects of the same disease. Thus, the previous caries experience variable and dental caries outcome might share the same or similar risk factors. We found this to be true in our analysis in the second manuscript (Chapter V). We assessed caries-related factors for cavitated caries occurrence between ages 5 and 9 years. In the univariable modeling, there was a strong relationship between caries occurrence and previous caries experience and weaker relationships between caries occurrence and certain other explanatory factors, such as SES and processed starches intake at snacks. In the multivariable logistic regression modeling, when a 0.05 significance level was applied in the backward elimination steps, dietary variables were not retained in the final model. This situation also has occurred in other published studies (Leroy et al., 2005; Tagliaferro et al., 2008). One study (Leroy et al., 2005), that conducted multivariable modeling including previous caries experience at age 6 and other related factors, reported that other modifiable factors that were significantly associated with new caries (between age 6 and 12 years) in the univariable analyses were not retained in the final model. Another study (Tagliaferro et al., 2008) examined children when they were 6 to 8 years old and again 7 years later. They discussed their findings that the oral hygiene habits variable was not significantly associated with caries incidence in the multivariable analysis because of the strong relationship between previous caries experience and caries incidence. Therefore, inclusion of previous caries experience in a multivariable model might reduce the contribution of the other factors. Thus, the other variables which have weaker relationships with dental caries would have been dropped out from the model. Thus, models with and without previous caries experience variables should both be considered, so that researchers can observe and compare the effects of previous caries experience on other caries-related factors. If substantial differences in the results are found, then the model that excluded the previous caries variable could be used to allow the researchers to identify a larger number of potential modifiable risk factors for caries.

The d/D component of dfs/DFS would be a better variable for previous caries experience than dfs/DFS itself, since the f/F component is sometimes a result of overtreatment of initial or arrested caries. However, in developed countries, the f/F component is found commonly among subjects. In the present study, more than 60% of children with some $d_{2+}f$ lesions had filled surfaces. Overall, about 70% of these $d_{2+}f$ surfaces were filled lesions. Therefore, exclusion of children with f/F component was not practical in this study.

Dietary Variables

Consumption of some dietary categories that were associated with a decreased likelihood of being in the caries group, but have no or little previous evidence as protective factors, might be indicators of other dietary consumption patterns. For example, the lower caries risk for children with high milk consumption at meals might be because of the physical properties of milk that could enhance remineralization and inhibit demineralization, or it might be because children with high milk consumptions at meals were less likely to have greater consumption of other beverages (replacement), or a combination of these two effects. Thus, recommendations to have more milk consumption at meals for preschool children would be expected to decrease non-cavitated caries in preschool children, regardless of the true reason(s) for the effect.

Published studies that assessed the associations between dental caries outcomes and dietary factors generally had "limited quality of dietary data" which impacts studies' results. Many studies that used data collected for other non-dental purposes did not distinguish snack vs. meal. Many studies used instruments for data collection that were not detailed enough for dental caries research. For example, food frequency questionnaires with only a few questions asking about specific foods and beverages are incomplete assessments of exposures and often miss key data. Also, many studies did not have a nutritional expert involved or had ones who were not oral health nutrition experts. The present study had an oral health nutritional expert.

Non-Cavitated and Cavitated Caries

More studies in the past 15 years used diagnostic criteria that differentiate non-cavitated caries from sound and cavitated caries surfaces, compared to older studies. However, only a small proportion of these have assessed caries-related factors for non-cavitated caries. There are no published studies that assessed factors associated with non-cavitated caries as part of the caries process by simultaneously and separately analyzing non-cavitated caries and cavitated caries outcomes in the same analyses. This project was the first to analyze factors related to these two outcomes separately in the same study.

There are two ways of analyzing both dental caries types. The first is using combined prevalence or incidence counts or a yes/no dichotomy for both non-cavitated and cavitated caries combined in one variable. One study (Bankel et al., 2006) categorized subjects with non-cavitated caries only, cavitated caries only or both caries types as cases for prevalence. There also are a few studies (Holbrook et al., 1995; Kolker et al., 2007) that summed non-cavitated lesions with cavitated caries using equal weights. Conceptually, researchers could weight non-cavitated lesions less than cavitated caries lesions, since cavitated lesions are a sign of a more advanced stage of the dental caries process. Filled lesions then could be weighted less heavily in some populations because filled lesions may represent over-treatment. Specifically, sometimes restorations may not be necessary; inactive lesions may be sealed instead of filled. At other times the number of filled surfaces exceeds the number of previously decayed surfaces. However, there are no published studies that have used such a weighted scheme for children. Since there have been a limited number of studies using equal weights and no studies using different weighting schemes, we cannot determine whether either one of them is more appropriate to assess caries risk factors.

A second approach is using categorical or ordinal caries outcomes by categorizing subjects into caries-free, non-cavitated caries only, cavitated caries only and both non-cavitated caries and cavitated caries groups. The first manuscript (Chapter IV) used this second method to analyze the data by examining caries risk factors for non-cavitated caries separately from cavitated caries. Using these 4 categories as an ordinal outcome would force the results to provide only a single set of associated variables modeled across all the groups. Moreover, it assumes that the relationships are the same between each ordered pair of adjacent categories (i.e., from the caries-free group to the d_1 group, the d_1 group to the $d_{2+}f$ group, or the $d_{2+}f$ group to the $d_1d_{2+}f$ group). There are no previously published studies that use this analysis method.

Regression Analysis vs. Other Approaches

All three manuscripts in this dissertation used regression analyses to assess associations between outcome variable(s) and multiple explanatory variables simultaneously, not just assessing differences of outcome among two or more independent groups. Moreover, regression analyses allow us to adjust for other covariates in the modeling and predict the outcome variable from the explanatory variables. For prospective epidemiological studies which cannot randomize or otherwise control for other factors in the design that might confound the associations among the outcome variable and the explanatory variables of interest, regression analyses are appropriate.

There are many regression methods that have been developed and used. The most common method is linear regression (often least squares regression) which is used to assess the association between normally distributed numerical outcomes and numerical explanatory variables (Dawson and Trapp, 2004). However, counts of caries surfaces, especially in low caries prevalence populations, usually are not normally distributed. Alternative methods for skewed numerical outcomes include Poisson, negative binomial, zero-inflated Poisson (ZIP) and zero-inflated negative binomial (ZINB) modeling. Negative binomial regression is an alternative method to Poisson regression. It can be used with discrete data over an unbounded positive range, and it allows sample variance to exceed the sample mean. ZIP and ZINB are intended for data with excess numbers of subjects with zero counts compared with those that are accounted for by the Poisson or negative binomial (Hilbe, 2007).

In the third manuscript (Chapter VI), the counts of new cavitated caries and counts of non-cavitated caries were used as the outcome variables. It was obvious that the data were not normally distributed, since there were a large number of children with zero newly affected surfaces. Results from exploratory analyses (data not shown) indicated that negative binomial and ZINB regressions (smaller AIC) fit the data better than did Poisson and ZIP. The results revealed that the data were fitted similarly well with both negative binomial and ZINB. There was no evidence of over-dispersion due to excess zero counts of the negative binomial regression. Therefore, negative binomial regression was chosen as the preferred method to use with our data.

With binary outcomes, logistic regression is commonly used to predict the probability of having disease. Logistic regression requires no distribution assumption. Regression coefficients from logistic regression are used to calculate odds ratios, which can be interpreted as estimates of the relative risk (risk ratio) of the outcome for those in one group vs. being in another group (Dawson and Trapp, 2004). The analysis in Chapter V that aimed to predict the probability of having new caries occurrence (yes/no) used this approach. Logistic regression also can be used with an outcome that has 3 or more categorical values, through ordinal (rank values) or multinomial (nominal values) regression. The analyses in Chapter IV assigned 4 groups for caries status as an outcome variable; thus, multinomial regression was used to assess the association between each group for caries status and the reference group.

Extra-Analyses

For the data concerning age 5 dental caries, we also explored the data using a few other types of analyses,including ANOVA and cluster analyses that are not shown in the main 3 chapters of this dissertation. We did those analyses for the purposes of exploring the data and learning about the approaches, but ultimately wedecided not to use those analyses in the dissertation manuscripts. ANOVA was the method that we used first with the dietary data from the questionnaires. We hypothesized that consumption frequencies of some beverage categories for the children in the 4 different caries groups were different. This analysis was bivariate and it did not control for other covariates.

Then, we started to use more detailed dietary information from the abstracted dietary diaries. We were interested in looking at the several dietary categories at the same time, so we conducted cluster analysis, which is a method that is used to group subjects with common characteristics together. Then, we compared the risk of having the disease among children in the different clusters. There are many types of clustering methods; one challenge with using cluster analysis is that different methods can yield different groupings (Templ et al., 2006). Moreover, specifications of the number of clusters and the variables used in the clustering procedure should be determined before conducting the cluster analysis. A second problem is that many techniques have been developed to determine the number of clusters, but there are no standard criteria for selecting the appropriate method. Third, sometimes cluster analysis resulted in a cluster with only a few subjects; thus, subjective decision-making was needed for combining such small clusters with other clusters. Additionally, there are quite a number of dietary categories involved in this analysis and we cannot include all variables in the cluster analysis. There are only a few studies concerning caries risk factors that used cluster analysis. Thus, the fourth problem is that we had no clear scientific methods that could be used in justifying variables for including in the cluster analysis.

Sohn et al. (Sohn et al., 2006) published findings from a study that investigated the effects of beverage consumption on caries using the NHANES III data. Cluster analysis was used to group subjects into 4 groups. The clustering procedure was based on the 4 primary sources of fluid consumption. However, they did not specify the criteria to identify “primary sources” or the reasons for using “four” primary sources in the clustering process. In addition, they determined the number of clusters for all subjects overall, but conducted the clustering procedure separately for each age group of 2-year-olds, 3- to 5-year-olds, and 6- to 10-year-olds. It is possible that the optimal numbers of clusters might be different for the different age groups.

Cross-Sectional Study vs. Prospective Study

Most of the studies assessing caries risk factors are cross-sectional studies. This type of study assesses the associations between caries-related factors and dental caries collected at the same time. The caries outcomes with this method are caries prevalence or counts of caries experience. The results of cross-sectional studies are considered as lower quality (lower research hierarchy) than those from cohort and experimental studies, since we do not know whether the caries-related factors preceded or followed the dental caries itself. For the analyses in Chapter V, we used the dental caries information from one dental examination at about age 5 which could be considered as lifetime (cumulative) caries prevalence from first tooth-eruption to about age 5. This analysis assessed the association between the lifetime (cumulative) caries prevalence and averages of repeated measures of explanatory variables from age 3 to 5. Even though the risk factors found associated with dental caries in the present analyses cannot be called causal factors, this analysis provides stronger evidence for the direction of the relationship and possibility of causality than factors found associated only in the cross-sectional studies because the age 3-5 exposure preceded the age 5 exam.

Randomized controlled trials (RCTs) usually considered the “best evidence”, especially for topics that lend themselves to controlling conditions that reduce the sources and/or magnitude of bias to enhance internal validity. However, an RCT for diet-related caries experience is not ethical due to potentially irreversible damage to the teeth. Also, it is difficult to expect to assign a full dietary intervention, with the complexity of dietary intakes. Thus, prospective cohort study data generally provide the best evidence available for assessing diet-related caries risk factors in humans. Larger cohort studies and the use of the multivariable regression analyses that can adjust for other variables are more appropriate for this type of caries research.

Analysis of Repeated Measurements

Repeated measures analysis theoretically has advantages over single outcome analysis. The analysis of repeated outcomes for individual subjects using appropriate statistical approaches would better control for individual differences, which increases the statistical power of inferential analyses. The analysis of repeated measurements also can assess the effect of risk factors jointly for the same subject over time. The analysis in the third manuscript (Chapter VI) is the first of its type to assess caries risk factors using analysis of repeated measurement with the caries outcomes from primary, mixed and early permanent dentitions. It could be used to find common caries-related factors that could be used in developing general preventive measures in children. Findings from the analysis indicated that greater consumption frequency of 100% juice was related to both fewer new non-cavitated and fewer new cavitated caries. However, consumption frequencies of other beverages, such as regular soda pop consumption, were not found to be significantly associated with either non-cavitated or cavitated caries, as we had found in the analyses in Chapter IV. Concerning age 5 caries outcomes, the lack of statistically significant association could be due to a power issue or because some dietary categories might be more important for individual time periods, but not for all ages. However, based on only the analysis in this dissertation, we cannot conclude that the repeated measures analysis is a better or worse way to assess caries-associated factors in children. We found that toothbrushing frequency decreased the risk of having non-cavitated caries across the three dentitions, which suggests that there might be some other possible risk factors that are more appropriate for this analytical approach across time periods. For example, water fluoride levels, topical fluoride exposures and socioeconomic status might be associated with caries outcomes for all age groups somewhat similarly. However, the wide variety of dietary intakes in children across ages might make them less appropriate for the repeated measures analysis. Moreover, dietary behaviors change with time and with introduction and availability of “new” foods. Thus, studies with very long follow-up times might not be a candidate for this approach._ Alternatively, the same major categories of factors might be consistently significantly related, but the significant subcategories might differ by age. Also, the same factors could be significantly related, but with different magnitudes of effect.

Skewed Outcome Variable (Many Cases with No Lesions)

In populations with low caries rates, the outcome for counts of carious surfaces has a large number of cases with no lesions. Thus, the distribution of the outcome variable would be a concern in choosing the analytical approach. There are several methods used in such studies in dental research, including modeling using the Poisson, negative binomial, zero-inflated Poisson, and zero-inflated negative binomial distributions. The analyses in Chapter VI used the counts of new caries surfaces as outcome variables (see more details in "Regression analysis vs. other approaches").

Future Research Directions

Future research studies should place more emphasis on investigation of risk factors for both non-cavitated and cavitated caries. A standard dietary categorization that could be used in studies that assess the relationships between diet and caries is needed. Findings from studies that used this standard dietary categorization could be compared more easily and could be used in developing clinical guidelines.

Moreover, research teams planning to conduct studies to assess risk factors for dental caries have to plan for large enough sample sizes to be able to use adjusted analyses to control for other covariates.

There are many characteristics of subjects that can affect the associations between dietary intake variables and caries outcomes. Populations with high access to care generally have more treated caries than do those with low access to care. Therefore, it is important to know whether dietary variables differently affect caries outcomes in populations with high vs. low access to care. Topical fluoride exposure could influence the associations to allow analyses between dietary intake variables and caries outcomes. Thus, more studies to investigate these associations between populations with high vs.

low access to care, and fluoridated vs. non-fluoridated drinking water, are needed. Food access also plays an important role in caries, since it affects food choices for people in the communities. Therefore, examining the effects of food access on caries could show useful findings to be considered in developing preventive measures.

It is also important to know whether different components of the caries outcome, including non-cavitated, cavitated and filled lesions, have similar associations with caries-related factors. Additional studies that assess and compare the use of equal weights vs. non-equal weights for cavitated, non-cavitated and filled lesions are also needed.

Summary and Clinical Implications

There are several factors such as previous caries experience and socioeconomic status that are known risk factors for caries. However, those are non-modifiable factors. The clinical implications for these variables are somewhat limited, since they cannot be changed. Thus, researchers should focus more on the modifiable factors that could be used in developing preventive guidelines or recommendations.

In the analyses in this dissertation, previous caries experience and SES had complex relationships with other independent variables. Including these variables in the multivariable analyses sometimes changed or weakened the associations between the caries outcomes and other variables.

Among the dietary intake variables, different variables were found to be significantly associated with dental caries in the primary and mixed dentitions. For the primary dentition examination at about age 5 years, greater regular soda pop consumption frequency at snacks (from ages 3 to 5 years) was a significant risk factor for cavitated caries in the primary dentition, but lower milk consumption frequency at meals and greater pre-sweetened cereal consumption frequency at meals (from ages 3 to 5 years) increased the risk of being in the d_1 group rather than in the caries-free group. For the age

5 to 9 caries outcome, greater processed starches consumption frequency at snacks (from ages 6 to 8 years) increased the probability of having new cavitated caries. The repeated measures analysis including age 5, 5 to 9 and 9 to 13 new caries experience indicated that greater consumption frequency of 100% juice was significantly associated with fewer new non-cavitated and fewer new cavitated caries surfaces across the primary, mixed and early permanent dentitions.

The findings in this analysis support the statement that "consumption of foods and beverages high in fermentable carbohydrates at meals is believed to decrease the cariogenicity of such consumption" in the published study (Marshall et al., 2005). The analyses in Chapter IV show that cavitated caries examination at age 5 ($d_{2+}fs > 0$) was significantly associated with the dietary consumption frequencies at snacks variables. Children with high consumption frequencies of either regular soda pop or added sugar at snacks were significantly more likely to be in the cavitated caries groups, which is the more advanced stage of dental caries. Results also show that non-cavitated caries was significantly associated with the dietary consumption frequency at meals variables. The greater consumption of pre-sweetened cereal and lower consumption of milk at meals were significantly associated with being in the d_1 group. At meals, foods and beverages are consumed with some other foods and/or beverages.

The analyses in Chapters IV and VI, which had both non-cavitated and cavitated caries as the outcome variables, consistently show that toothbrushing frequency was significantly associated with non-cavitated caries. However, toothbrushing frequency was significantly associated with cavitated caries in the analysis in Chapter V (for age 5-9 caries incidence), but was not statistically significantly related in the analyses in Chapters IV (age 5 caries) and VI (ages 5, 5-9 and 9-13 caries). Additionally, since more than 99% of the children in this study used fluoridated toothpaste, the toothbrushing variable really was a measure of frequency of topical fluoride exposure and mechanical cleaning combined.

Conclusions

The results from these three manuscripts together lead to the following conclusions:

1. Different dietary variables were significantly associated with dental caries outcomes for different age groups, possibly due to differences in the definitions of the variables, analytical approaches, and dietary consumption patterns, as well as sample size considerations across the three major analyses.
2. Consumption of foods or beverages at meals generally was more weakly associated with caries outcomes than was consumption at snacks.
3. Previous caries experience often weakened the associations between caries outcomes and other explanatory variables in the regression models. This could result in some modifiable variables not being found to be statistically significantly related, including dietary variables, which usually had less strong associations with dental caries.
4. Approaches using the analysis of repeated measurements might be more appropriate for variables that are common and fairly consistent in all age groups, such as toothbrushing frequency and fluoride exposures, or with those adult populations who have stable dietary intake patterns.

APPENDIX A

POSTSCRIPT: AGE 5 EXTRA ANALYSES

Dental Caries at Age 5

Objective

This descriptive analysis was an intermediate step that aimed to present detailed information on dental caries experience of all children who were examined for caries status at about age 5. In the main results presented on the age 5 data (Chapter 4), dental caries analyses were contributed with the smaller sample size.

Results

Six hundred and ninety-eight children were examined for primary dentition caries status. The mean age at dental exam was 5.17 (±0.42) years. Considering only cavitated caries experience (cavitated caries (d_{2+}) and filled (f) tooth), 27.4% of the children had caries experience (Warren et al., 2002). Considering cavitated, filled and non-cavitated (d_1) caries, 37.1% of the children had caries. The details of the prevalence and mean numbers of surfaces with specific caries categories d_1, d_{2+}, f, d_{2+}f and d_1 d_{2+}f are presented in Table A1.

Each child was classified based on the dental caries status of all teeth into 8 person-level caries experience groups reflecting all possible combinations of all caries components. Table A2 shows the distribution of children in these 8 groups. About 63% of children were caries-free. About 5% of children had all of d_1, d_{2+} and f caries. Table A3 shows the distribution of children collapsed into 4 person-level caries groups which, were sound, d_1 only, d_{2+}/f only and d_1 and d_{2+}/f groups.

Based on these 4 person-level caries groups, the mean numbers of surfaces of all caries components among children within person-level caries group were calculated and are presented in Table A4. The last two columns show mean scores by two different weighting schemes for the caries components. The first weighting scheme weighted d_1 lesions as 0.50 and filled lesions as a 0.75 of d_{2+} lesions. The second weighting scheme weighted d_1 lesions as a 0.25 and filled lesions as 0.50 of d_{2+} lesions.

Table A1 Prevalence and mean numbers of surfaces with specific caries categories

	Prevalence (%)	Mean surfaces (all subjects)	Mean (only subjects with specific caries)
Non-cavitated caries (d_1)	24.2	0.66	2.43
Cavitated caries (d_{2+})	16.5	0.44	2.66
Filled caries (f)	17.5	1.04	5.96
Cavitated caries experience (d_{2+}f)	27.4	1.48	4.74
All caries experience (d_1 d_{2+}f)	37.1	2.14	8.91

Table A2 Distribution of children by 8 groups of person-level caries experience

Group	N	Percent
Sound	439	62.9
d_1 only	68	9.7
d_{2+} only	28	4.0
filled only	48	6.9
d_1 and d_{2+}	41	5.9
d_1 and filled	28	4.0
d_{2+} and filled	14	2.0
d_1, d_{2+} and filled	32	4.6
Total	698	100

Table A3 Distribution of children by 4 groups of person-level caries experience

Group	N	Percent
1.Sound	439	62.9
2. d_1 only	68	9.7
3. d_{2+}/f only	90	12.9
4. d_1 and d_{2+}/f	101	14.5

Table A4 Mean (median) carious lesions by 4 person-level caries groups (n=698)

Caries group	d_1	d_{2+}	f	d_1 d_{2+}	d_{2+}f	d_1 d_{2+}f	weight1*	weight2**
Sound (n=439)	0	0	0	0	0	0	0	0
d_1 only (n=68)	2.43 (2.00)	0 (0)	0 (0)	2.43 (2.00)	0 (0)	2.43 (2.00)	1.21	0.61
d_{2+}f only (n=90)	0 (0)	1.19 (0.00)	3.56 (2.00)	1.19 (0.00)	4.74 (2.00)	4.74 (2.00)	3.86	2.97
d_1 and d_{2+}f (n=101)	2.91 (2.00)	1.97 (1.00)	4.03 (1.00)	4.88 (4.00)	6.00 (4.00)	8.91 (6.00)	6.45	4.71

Note: * $d_1 = 0.5$, $f = 0.75$, $d_{2+} = 1$, ** $d_1 = 0.25$, $f = 0.5$, $d_{2+} = 1$

Questionnaire Analyses

Objective

This analysis examined the relationships between person-level caries status categories at 5 years old and socioeconomic status at recruitment, frequencies and quantities of intake from different beverage categories, and home water fluoride levels from birth to 60 months old.

Methods

Inclusion in analyses required a minimum of 7 out of 16 possible questionnaires from birth to 60 months old (n=606). Beverage consumption data from questionnaires—milk, ready-to-drink juices/juice drinks, non-juice beverages (excluding water/milk), and beverages from powdered concentrates—and home water fluoride levels were averaged from birth to 60 months. Children were categorized into 4 groups based on individual caries experience.

Group A -"sound only" (caries-free),

Group B -"most involved was non-cavitated caries" (d_1),

Group C -"only cavitated and/or filled caries" ($d_{2+}f$), and

Group D -"both non-cavitated and cavitated and/or filled caries" ($d_1d_{2+}f$).

Statistical analyses included ANOVA and Chi-square tests.

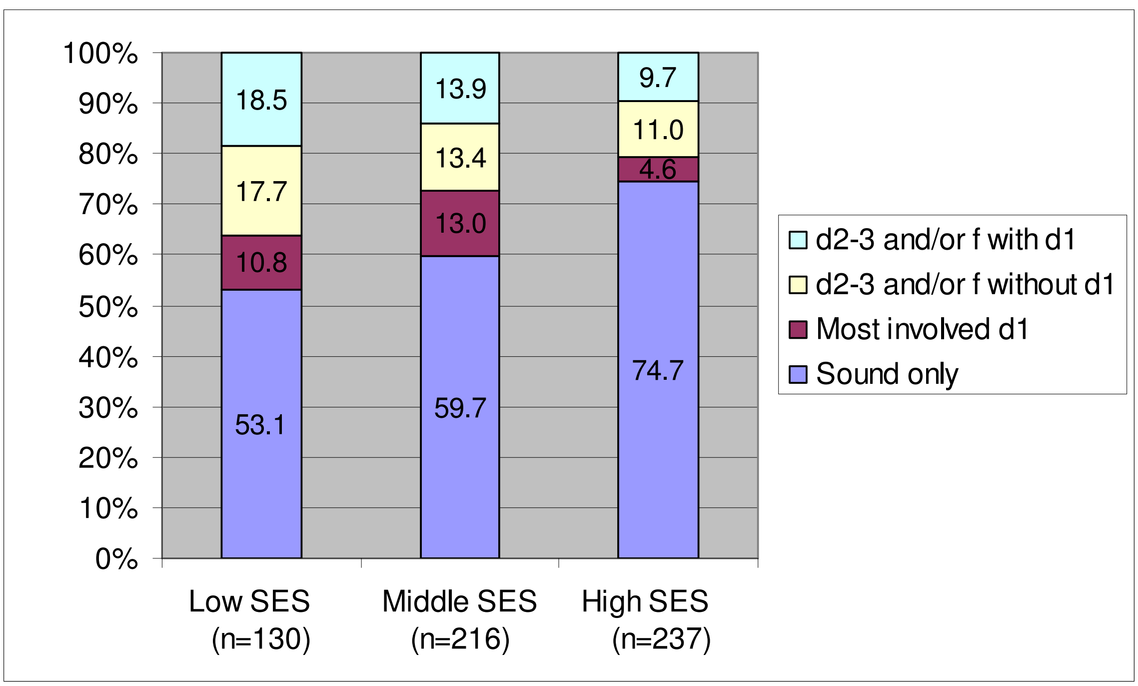
Results

There were 606 children included in the analyses. Distribution of children in person-level caries experience categories by SES levels is presented in Figure A1. Socioeconomic status ($p<0.001$) and home water fluoride levels ($p=0.01$) were significantly associated with the dental caries category. Subject group by type of caries was associated with mean frequency and quantity of beverage intakes for: ready-to-drink juices and juice drinks (both $p<0.001$), non-juice beverages (both $p<0.05$), and beverages from powdered concentrates (both $p<0.005$) (Table A5). There was a generally consistent trend for beverage intakes, including ready-to-drink juice/juice drinks, non-juice beverages and beverages from powdered concentrates, and caries outcomes; individuals with less severe lesions had lower frequencies and quantities of intakes (caries free < d_1 only< $d_{2+}f$ only < $d_1d_{2+}f$, with $d_1d_{2+}f$ most dissimilar).

Limitations

This analysis was one step of the learning process. The analysis was based on the data from the IFS questionnaires. The questionnaires were generally less time-consuming for subjects, thus we have a very high response rate. However, the IFS questionnaires were originally designed primarily for collecting information, especially water quantities, to estimate amounts of fluoride intake. Data of beverage intakes from questionnaires did not separate sugar-free beverages from sugared beverages and 100% juice from juice drinks. Thus, the findings from the analyses using this data might not be fully explained for the purpose of the assessment of the associations between caries and beverage intakes.

Figure A1 Percentages of children in person-level caries experience categories by SES levels (n=583)



Note**:** Chi-Square test shows significant association between SES level and person-level caries experience (p=0.0005) (data missing in 23 subjects)

Table A5 Comparison of daily mean[a] beverage drinking events and quantities by person-level caries experience category.

Beverages	Type of variable	Group A Sound only (N=387)	Group B d_1 only (N=59)	Group C d_{2+} and/or f without d_1 (N=80)	Group D d_{2+} and/or f with d_1 (N=80)	p-value (ANOVA)	Tukey's Post Hoc Tests (Significant pairwise different shown)
Milk	Frequency	10.55	9.87	9.86	9.31	0.0827	
	Quantity (ounces)	5.64	5.78	5.60	5.37	0.6984	
RTD juices/juice drink	Frequency	3.42	3.43	4.71	4.75	0.0001[c]	A-D, A-C
	Quantity (ounces)	2.80	2.72	3.38	3.38	0.0008[c]	A-D, A-C
RDT Non-juice beverages (excluding milk and water)[b]	Frequency	1.42	1.34	1.31	1.96	0.0093[c]	A-D, C-D
	Quantity (ounces)	2.04	2.10	2.23	2.48	0.0497[c]	A-D
Beverages from powdered concentrates [b]	Frequency	1.17	1.61	1.86	2.55	<0.0001[c]	A-D
	Quantity (ounces)	1.21	1.42	1.46	1.77	0.0020[c]	A-D
Combined of RTD juices/juice drink, non-juices drinks, and beverages from powdered concentrates [b]	Frequency	6.47	6.89	8.40	10.07	<0.0001[c]	A-C, A-D, B-D
	Quantity (ounces)	6.03	6.26	7.03	7.63	<0.0001[c]	A-C, A-D, B-D

[a] Mean of all responses to questionnaires from 1.5 to 60 months

[b] Diet beverages included with sugar beverages in questionnaire # 8.10 and 8.13

[c] Statistically significant

Dietary Diary Analyses

Objective

This analysis examined the relationships between person-level caries status categories at 5 years old and socioeconomic status at recruitment, quantities of intake from different beverage categories assessed by diaries, and home water fluoride levels from birth to 60 months old.

Methods

Three-day dietary diaries were used to quantify beverage intake data. Inclusion in analyses required a minimum of 7 out of 16 possible dietary diaries from 1.5 to 60 months old (n=529). Averaged daily consumptions of each beverage category including unsweetened whole milk, sweetened milk, unsweetened low fat milk, juice drinks, 100% juice, regular (sugared) soda pop, sugar-free soda pop, sugared beverages reconstituted from powder, sugar-free beverages reconstituted from powder, and water and home water fluoride levels were calculated from all available diaries from 1.5 to 60 months. Children were categorized into 4 groups based on individual caries experience.

Group A -“sound only” (caries-free),

Group B -“most involved non-cavitated caries” (d_1),

Group C -“only cavitated and/or filled caries” ($d_{2+}f$), and

Group D -“both non-cavitated and cavitated and/or filled caries” ($d_1d_{2+}f$).

ANOVA was used in these analyses.

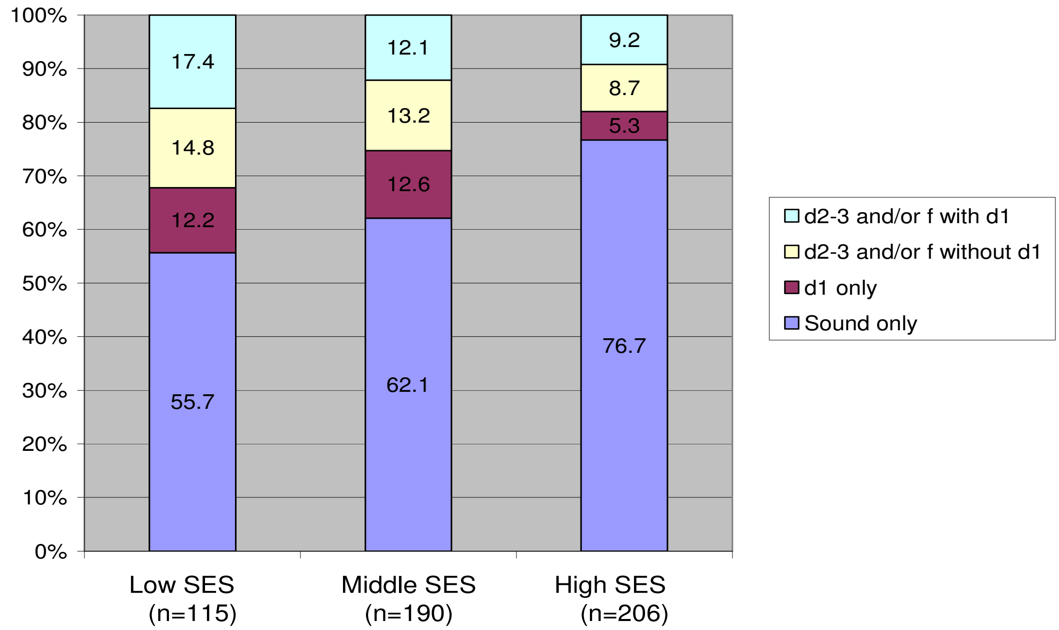
Results

There were 529 children included in the analyses. Socioeconomic status category ($p<0.005$) was significantly associated with the caries groups (Figure A2). Mean intakes of sugared soda pop and sugared beverages from powdered were significantly different among children in the different caries groups. Results from Tukey’s Post Hoc Tests indicated that subjects who had d_1 and $d_{2+}f$ caries had significantly greater daily intakes of sugared soda pop ($p<0.005$) and sugared beverages reconstituted from powder intakes ($p<0.005$) than did subjects who had no caries (Table A6). No significant differences of means daily intake amounts of sugar-free beverages, milk, juice drinks and juice were found among children in the different caries groups.

Limitations

This analysis was also one step of the learning process. The diet diaries provided better information, but a smaller sample size compared with the analyses using the questionnaire data. There were fewer subjects who had dietary diary data than those who had questionnaire data because the diaries were more time-consuming than the IFS questionnaires. This analysis included the subjects who had at least 7 diaries out of 16, without regard to which ones, and simply average then at any time-point. Thus, it was possible that some children might have had beverage intake data more from earlier time-points, while some children might have had beverage intake data more from later time-points. Efforts to require more diaries total for each subject or require one diary per year meant be much lower sample sizes. Moreover, the analysis was bivariate and did not adjust for other variables.

Figure A2 Percentages of children in person-level caries experience categories by SES levels (n=511)



Note: Chi-Square test (using Ridit Score) shows significant association between SES category and person-level caries experience ($p<0.0001$) (data missing for 18 subjects)

Table A6 Comparisons of mean daily beverage intakes (ounces) by person-level caries experience category.

Beverages	A. Sound only (N=350)	B. d_1 only (N=53)	C. d_{2+} and/or f without d_1 (N=62)	D. d_{2+} and/or f with d_1 (N=64)	p-value (ANOVA)	Tukey's Post Hoc Tests (Significant pairwise different shown)
Unsweetened Whole Milk	2.65 (±2.91)	2.73 (±3.05)	2.44 (±2.47)	2.63 (±2.97)	0.96	
Sweetened Milk	0.47 (±1.18)	0.62 (±1.57)	0.54 (±0.88)	0.26 (±0.43)	0.34	
Unsweetened Low Fat Milk	6.10 (±4.01)	5.47 (±4.39)	6.21 (±3.83)	5.82 (±4.36)	0.71	
All Milk[a]	9.23 (±4.10)	8.82 (±4.40)	9.19 (±3.53)	8.71 (±3.93)	0.76	
Juice Drinks	1.25 (±1.41)	1.46 (±1.96)	1.11 (±1.29)	1.01 (±1.01)	0.34	
100% Juice	3.46 (±2.23)	3.26 (±1.96)	3.90 (±2.47)	3.60 (±3.13)	0.47	
Regular (Sugared) Soda Pop	0.85 (±1.03)	0.89 (±0.83)	1.18 (±1.17)	1.32 (±1.26)	0.0029	A-D
Sugar-free Soda Pop	0.22 (±0.51)	0.23 (±0.56)	0.22 (±0.47)	0.24 (±0.46)	0.99	
Sugared Beverages Reconstituted from Powder	0.53 (±1.11)	0.89 (±1.69)	1.02 (±1.77)	1.02 (±1.69)	0.0035	A-C, A-D
Sugar-free Beverages Reconstituted from Powder	0.07 (±0.64)	0.04 (±0.20)	0.10 (±0.37)	0.15 (±0.83)	0.75	
Water	3.23 (±2.90)	3.21 (±2.61)	2.96 (±2.37)	2.89 (±2.77)	0.77	
Added Sugar Beverages[b]	3.09 (±2.76)	3.85 (±3.40)	3.61 (±3.13)	3.85 (±2.54)	0.076	
Sugar-free Beverages[c]	0.29 (±0.88)	0.27 (±0.62)	0.32 (±0.58)	0.39 (±0.95)	0.83	

[a]Excluding breastfeeding and infant formulas

[b]Sugared soda pop, juice drinks, sugared beverages reconstituted from powder and sweetened milk

[c]Sugar-free soda pop and sugared beverages reconstituted from powder

Cluster Analysis (Part I)

Objective

These analyses aimed to assess the relative risk of having d_1 caries only and relative risk of having $d_{2+}f$ caries at age 5 associate with different beverage intake patterns at 36, 48 and 60 months old.

Methods

The most common dietary factors which were significantly associated with person-level caries experience groups among the 3 time points from ANOVA tests were included in the analyses. A principal component analysis was used to centralize means of the variables and make the data ready for the clustering procedure. SAS was used to create clusters. Four variables were included in the clustering procedure. The R-square and Pseudo F statistics were calculated. Based on these results, four clusters were used for grouping the children. The relative risks of having d_1 and $d_{2+}f$ caries for each intake pattern were then calculated.

Results

There were 453 children who had dietary intakes data at age 36 months and dental caries data at age 5. Juice drink intake at snack time, powdered sugared beverages intake at snack time, regular soda pop intake at snack time and sugars and starches intake at meal times were selected for the clustering process. The children were clustered into 4 groups based on their intake patterns of these four categories. These were called low sugared beverage intake at snack times, high juice drink intake at snack times, high powdered sugared beverage intake at snack times and high regular soda pop intake at snack times. The mean intakes for the 4 dietary variables for children in each cluster are presented in Table A7.

Table A8 shows the distribution of children in 3 caries groups (sound, d_1 only and $d_{2+}f$ (with or without d_1)) by the 4 clusters. Then the relative risk of having d_1 and relative risk of having $d_{2+}f$ (with or without d_1) are presented in Table A9. The children in the high regular soda pop intake at snack times cluster had 2.16 (1.47, 3.19) times the risk of having $d_{2+}f$ caries as the children in the low sugared beverages intake at snack times the risk of cluster, while the children in the high juice drink intake at snack time cluster had 2.30 (1.02, 5.22) times of having d_1 only caries as the children in the low sugared beverages intake at snack times cluster. Other comparisons were not statistically significant, but consistent in overall pattern.

The traditional approach of considering only cavitated caries as a case was used and relative risk of having $d_{2+}f$ caries (compared to non-case which combined sound and d_1 only together) was calculated and is presented in Table A10. The children in high regular soda pop intake at snack times cluster had 2.12 (1.42, 3.19) times the risk of having $d_{2+}f$ caries as the children in the low sugared beverages intake at snack times cluster, with the other 2 comparisons not statistically significant.

The results of age 48 and 60 months old analyses are not shown in the dissertation.

Limitations

These analyses examined not one dietary variable at a time, but the intake patterns of the key variables at each time point. However, cluster analysis is not a common method that has a solid methodology. The criteria used to select variables from all available variables were set by the author. The number used to set how many clusters wanted in the analysis was based on 1) adequate sample size in each cluster and 2) the obvious characters of clusters that could be explained clinically. So, some might question the soundness of the method used in these analyses.

Table A7 Mean intake of foods and beverages at 36 months by 4 clusters

Variable	Low sugared beverages at snack time (n=369)	High juice drink at snack time (n=19)	High powdered beverage at snack time (n=27)	High pop at snack time (n=38)
Juice drink at snack time (ounces)	0.35	7.36	0.33	0.59
Powdered sugared beverage at snack time (ounces)	0.40	0.67	9.32	0.47
Regular soda pop at snack time (ounces)	0.16	0.67	0.54	4.53
Sugar and starch at meal time (occasions)	4.20	4.69	4.09	4.01

Table A8 Distribution of children into different person level caries groups at age 5 by 4 clusters of food and beverage intakes at age 36 months

Caries group	Cluster				Total
	Low sugared beverages at snack time	High juice drink at snack time	High powdered beverage at snack time	High pop at snack time	
Sound	256 (69.95%)	14 (51.85%)	9 (50.00%)	17 (44.74%)	296 (65.92%)
d_1 only	33 (9.02%)	5 (18.52%)	3 (16.67%)	4 (10.53%)	45 (10.02%)
$d_{2+}f$ (with or w/o d_1)	77 (21.04%)	8 (29.63%)	6 (33.33%)	17 (44.74%)	108 (24.05%)
Total	366 (81.51%)	27 (6.01%)	18 (4.01%)	38 (8.46%)	449 (100%)

Table A9 Relative risk of having caries by cluster (4 clusters of dietary intake at 36 months)

Variable	Cavitated caries (with or w/o d_1)			
	n**	Percent cavitated caries	Relative risk	95% CI
Low sugared beverages at snack time (Ref)	333	23.12%	1.00	
High pop at snack time	34	50.00%	2.16*	1.47, 3.19
High juice at snack time	22	36.36%	1.57	0.87, 2.83
High powdered beverages at snack time	15	40.00%	1.78	0.90, 3.31
Variable	Non-cavitated caries (without $d_{2+}f$)			
	n***	Percent Non-cavitated caries	Relative risk	95% CI
Low sugared beverages at snack time (Ref)	289	11.42%	1.00	
High pop at snack time	21	19.05%	1.67	0.65, 4.26
High juice at snack time	19	26.32%	2.30*	1.02, 5.22
High powdered beverages at snack time	12	25.00%	2.19	0.78, 6.14

* Statistically significant difference from 1

** Smaller n because excluded d_1 only people

*** Smaller n because excluded those with $d_{2+}f$

Table A10 Relative risk of having caries by cluster (4 clusters of dietary intake at 36 months) (No disease including sound only and d_1 only)

Variable	Cavitated caries (with or w/o d_1)			
	n	Percent cavitated caries	Relative risk	95% CI
Low sugared beverages at snack time (Ref)	366	21.04%	1.00	
High pop at snack time	38	44.74%	2.12*	1.42, 3.19
High juice at snack time	27	29.63%	1.41	0.76, 2.60
High powdered beverages at snack time	18	33.33%	1.58	0.80, 3.14

* Statistically significant difference from 1

Cluster Analysis (Part II)

Objective

This analysis aimed to examine the association between the intake patterns of specific beverages over time and the risk of having d_1 and $d_{2+}f$ caries.

Methods

The regular soda pop intakes at 36, 48 and 60 months old were used in this analysis. A principal component analysis was used to centralize means of the variables and make the data ready for clustering procedure. The R-square and Pseudo F statistics were calculated. The number used to set how many clusters wanted for the analysis was based on the graphs of the R-square and Pseudo F statistics (Figures A3 and A4). Five clusters were suggested for the cluster analysis procedure. The outcome of five clusters was used for grouping the children. The distribution of children in each caries group—sound only, d_1 only and any $d_{2+}f$ group—was assessed and the relative risks of having d_1 and $d_{2+}f$ caries for each regular pop intake pattern were then calculated.

Results

There were 354 children in this analysis. They were grouped into 5 clusters (Figure A5). There were 20 children in cluster 1 in which the children had high intakes of regular soda pop at all 3 time-points. There were 208 children in cluster 2 in which the children had low intakes of regular soda pop at all 3 time-points. There were 29 children in cluster 3 in which the children had high intake of regular soda pop at 36 months, but low intakes of regular soda pop at 48 and 60 months. There were 57 children in cluster 4 in which the children had high intake of regular soda pop at 48 months, but low intakes of regular soda pop at 36 and 60 months. There were 40 children in cluster 5 in which the children had high intake of regular soda pop at 60 months, but low intakes of regular soda pop at 36 and 48 months. Table A11 and Figure A4 show mean regular soda pop intakes of children in each cluster at 36, 48 and 60 months old.

Table A12 shows the distribution of children into 3 caries groups (sound group, d_1 only group and any $d_{2+}f$ group) by cluster. Cluster 1 had the highest percentage (35%) of children in the any $d_{2+}f$ group, while cluster 2 had the lowest percentage (20.2%) of children in the any $d_{2+}f$ group. Among clusters 3, 4 and 5, which each had only one of the 3 time points that had high intake of regular soda pop; they had similar percentages of children in the sound group. However, the closer the time period during which they had high intake of regular soda pop to the exam, the higher the percentage of children in the any $d_{2+}f$ group.

Limitations

This analysis was better in term of clustering procedure; however, this approach cannot be use with the repeated measures of several independent variables, since it is too complicate for the clustering procedure. The higher the number of variables included in the cluster analysis, the greater the possibility that the number of clusters suggested will be higher. Therefore, it is possible that the number of children in some clusters will be very small and the sample size will be too small to do statistical tests or to have reasonable power. Moreover, with large number of clusters used in the clustering procedure, some clusters would have small differences of independent variables, thus it is difficult to clinically describe characteristics of subjects in each cluster.

Figure A3 Number of clusters for regular soda pop intake at 36, 48 and 60 months old (R-square)

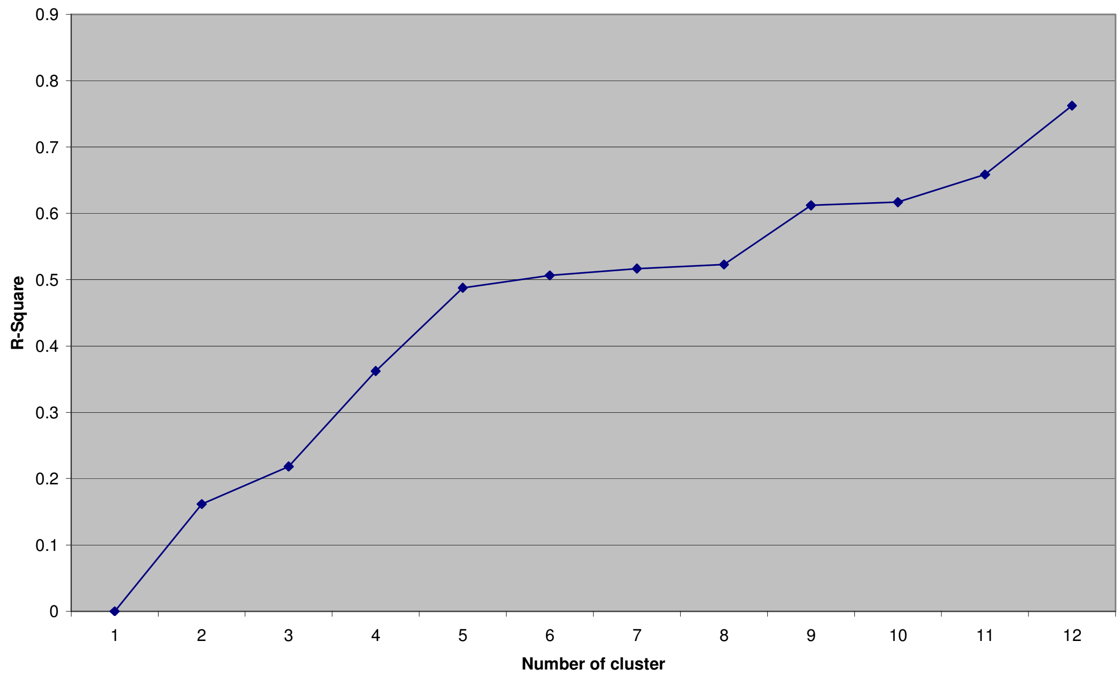


Figure A4 Number of clusters for regular soda pop intake at 36, 48 and 60 months old (Pseudo F statistic)

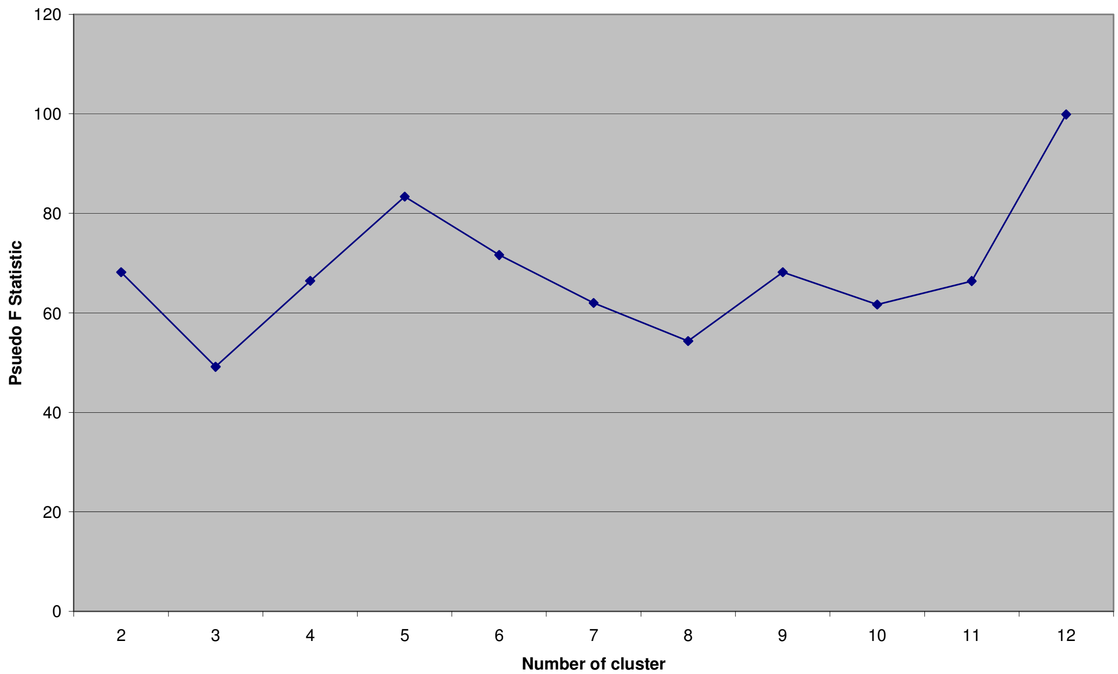


Figure A5 The patterns (mean intakes) of regular pop intake at 36, 48 and 60 months old by 5 clusters

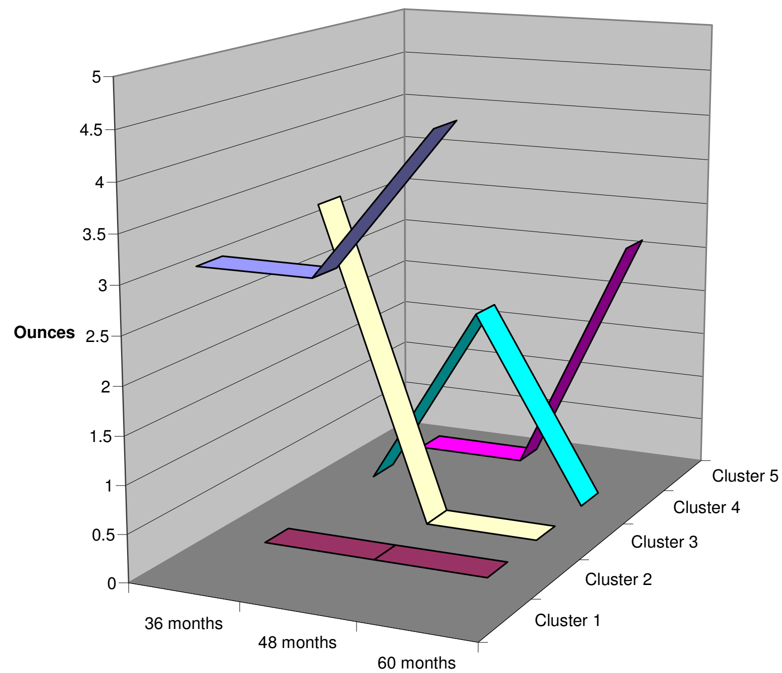


Table A11 Mean intakes of regular pop intake at 36, 48 and 60 months old by 5 clusters

Time-point	Median (mean) Intake (Ounces)				
	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
	High at all 3 time-points	Low at all 3 time-points	High at 36 months but not 48 and 60 months	High at 48 months but not 36 and 60 months	High at 60 months but not 36 and 48 months
36 months	3.17 (3.05)	0.00 (0.09)	3.33 (3.94)	0.00 (0.34)	0.00 (0.14)
48 months	3.17 (3.40)	0.00 (0.03)	0.00 (0.18)	2.00 (2.63)	0.00 (0.25)
60 months	4.67 (4.38)	0.00 (0.08)	0.00 (0.48)	0.00 (0.34)	2.67 (3.19)

Table A12 Distribution of children in caries groups by clusters

Caries Group	Cluster					Total
	Cluster 1 High at all 3 time-points	Cluster 2 Low at all 3 time-points	Cluster 3 High at 36 months but not 48 and 60 months	Cluster 4 High at 48 months but not 36 and 60 months	Cluster 5 High at 60 months but not 36 and 48 months	
Sound	11 55.00%	145 69.71%	18 62.07%	35 61.40%	25 62.50%	234
d_1 only	2 10.00%	21 10.10%	4 13.79%	6 10.53%	3 7.50%	36
Any $d_{2+}f$	7 35.00%	42 20.19%	7 24.14%	16 28.07%	12 30.00%	84
Total	20	208	29	57	40	354

APPENDIX B

IOWA FLUORIDE STUDY QUESTIONNAIR I

For Age 2 to 8.5 Years Old Children

IOWA FLUORIDE STUDY

COLLEGE OF DENTISTRY
THE UNIVERSITY OF IOWA

24 MONTH QUESTIONNAIRE

Please review the address label and make any necessary corrections:

PLEASE DO NOT REMOVE THIS PAGE

DIRECTIONS

A. Please circle the number beside the response that best matches your answer.

For example: Did your child take any fluoride drops or tablets during the last 4 months?

1 yes
2 no

If you are unsure of your answer, you may write your comments in the margin.

B. For some questions you will need to write your answer in the available boxes.

For example: Please write in today's date: |__|__| |__|__| 19|__|__|
month day year

C. It is very important that you fill out your child's **weight**. If the weight from a doctor's visit is not available, then use a bathroom scale.

D. Please write in the brand names of products when requested.

E. If you have any questions, please call Mary Kiritsy, the project coordinator **collect** at (319) 335-7182 or the Iowa Fluoride Study Team office **collect** at (319) 335-7026.

We appreciate your participation and thank you for completing this questionnaire.

IOWA FLUORIDE STUDY
24 MONTH QUESTIONNAIRE

PLEASE START HERE

OFFICE USE ONLY:
SUBJECT ID: |___|___|___|___|
QUESTIONNAIRE ID: | 0 | 8 |
DATE: |__|__| |__|__| |__|__| (month / day / year)

1. Please write in today's date: |___|___| |___|___| 19|___|___| (month / day / year)

2. How much does your child weigh? |___|___| lbs
[If your child's weight from a doctor's visit is not available, please use a bathroom scale]

3. How tall is your child? |___|___|.|___|___| inches **[½" = .50"]**

4. Have you moved in the last 4 months?

   1 yes **[Please make address corrections to label on page 1]**
   2 no

5. Where does the water from your kitchen faucet or tap come from?

   1 public or city water supply
   2 private or individual well
   3 community well, such as a mobile home park or subdivision with its own well
   4 not sure (please explain)

6. Do you use a water filtration or purification system with the water from your kitchen faucet or tap? This would **usually** be either attached directly to the faucet or installed beneath the sink and does not include a water softener.

   1 yes
   2 no **[SKIP TO QUESTION # 7]**

   6a. Do you use the following types of filtration systems:

   6a.1 reverse osmosis? 1 yes 2 no
   Please list brand and model ____________________

   6a.2 distillation system? 1 yes 2 no
   Please list brand and model ____________________

   6a.3 charcoal/carbon filter? 1 yes 2 no
   Please list brand and model ____________________

   6a.4 other? 1 yes 2 no
   Please list brand and model ____________________

7. At home, during the last week, did your child have any water?
Include water by itself, water used to make beverages, food, etc.

1 yes
2 no **[SKIP TO QUESTION # 8]**

7a. Was the water mostly tap water from your kitchen faucet or bottled water (including water from the water dispenser, jugs or bottles from the grocery store or water delivered to your home)?

1 mostly tap water.

2 mostly bottled water.

3 about equal amounts of both tap and bottled water.

7b. If using any **bottled water** please list brand name(s) and/or supplier(s).

____

The following question is about all the food and beverages that your child ate and drank during the last week.

8. Please complete the table by circling **YES** or **NO** if the food or beverages were eaten by your **NUMBER OF SERVINGS** your child ate and drank **DURING THE LAST WEEK** and the **AMOUNT EATEN PER SERVING.**

EXAMPLE:	**YES NO** **1 2**	**NO. OF SERVINGS PER WEEK**	**AMOUNT PER SERVING**
8. Ready-to-feed juice and juice drinks drank as purchased. **Type(s)/Brand(s):**	YES NO	|___|____|	|__|__|oz

Please note that some questions have now been deleted.

WATER AND BEVERAGES AT HOME, CHILD CARE, ETC. **DURING THE LAST WEEK**	**YES 1**	**NO 2**	**NO. OF SERVINGS PER WEEK**	**AMOUNT PER SERVING**
1. Water *(by itself, not mixed with anything).*	YES	NO	|____|____|	|___|___|oz
5. Milk *(including chocolate milk, eggnog).*	YES	NO	|____|____|	|___|___|oz
8. Ready-to-feed juices and juice drinks drank as purchased. **Type(s)/Brand(s):**	YES	NO	|____|____|	|___|___|oz
9. Ready-to-feed juices you diluted. **Type(s)/Brand(s):** ____________________ 9a. Circle the dilution used: 1. ¾ juice/ ¼ water 2. ½ juice/ ½ water 3. ¼ juice/ ¾ water	YES	NO	|____|____|	|___|___|oz
10. Non-juice beverages drank as purchased *(carbonated beverages, Pedialyte®, etc.).* **Type(s)/Brand(s):**	YES	NO	|____|____|	|___|___|oz
11. Beverages mixed as directed from frozen concentrate *(3:1 for juice drinks, etc. and 4 1/3:1 for lemonade).* **Type(s)/Brand(s):**	YES	NO	|____|____|	|___|___|oz
12. Beverages from frozen concentrate you diluted beyond package directions. **Type(s)/Brand(s):**	YES	NO	|____|____|	|___|___|oz
13. Beverages from powdered concentrate *(Kool-aid®, etc.)* and coffee and tea. **Type(s):**	YES	NO	|____|____|	|___|___|oz
FOODS AT HOME, CHILD CARE, ETC. **DURING THE LAST WEEK**			**8oz= 1 cup**	**4oz=½cup**
16. Foods made with all water *(Jello®, dry soup, etc.).* **Type(s):**	YES	NO	|____|____|	|___|___|oz
17. Foods made with some water *(canned soup, etc.).* **Type(s):**	YES	NO	|____|____|	|___|___|oz
18. Foods cooked in water *(rice, macaroni, hot cereal etc.).* **Type(s):**	YES	NO	|____|____|	|___|___|oz

The following questions are about your child's child care:

9. During the last 4 months, has your child attended child care outside your home?

   1 yes
   2 no **[SKIP TO QUESTION # 17]**

10. During how many weeks in the last 4 months (17 weeks) did your child attend child care?

    |___|___| weeks

11. During the weeks that your child attended child care, how many days (full days or half days)

    |_____| days per week

12. Were the child care days during the last 4 months typically:

    1 mostly full days (5 hours or more)
    2 mostly half days (3-4 hours)
    3 other (please explain) ______________________________________

13. On child care days, during the last 4 months did your child have any water (including water by itself, as well as water added to beverages and foods) **from the child care source?**

    1 no water from the child care source **[SKIP TO QUESTION # 17]**.
    2 some or all water from child care source.

14. Please list the amounts of water your child drank on an average day **from the child care source**.

CHILD CARE DAY	WATER BY ITSELF	WATER ADDED TO FOOD AND BEVERAGES
8 oz = 1 cup 4 oz = ½ cup	ounces/day	ounces/day
3. *Water from child care*	|___|___|oz	|___|___|oz

The following questions are about the fluoride level of the water at child care:

16.1 Is the tap water at child care from:

   1 public or city water supply
   2 private or individual well
   3 community well, such as a mobile home park or subdivision with its own well

16.2 In which city is your child care located? ________________________

16.3 Is the tap water at child care filtered?

1 yes
2 no **[SKIP TO QUESTION # 17]**

16.3a Which of the following types of filtration systems is used at child care?

16.3a.1	reverse osmosis? **Please list brand and model**	1 yes	2 no
16.3a.2	distillation system? **Please list brand and model**	1 yes	2 no
16.3a.3	charcoal/carbon filter? **Please list brand and model**	1 yes	2 no

The following question is about your child's feeding habits:

17. Were your child's feeding habits during the last week representative of his or her feeding habits <u>during the last 4 months</u>?

1 yes **[SKIP TO QUESTION # 18]**
2 no, there were changes in feeding habits.

FEEDING HABIT CHANGES	**YES 1**	**NO 2**
17a.5 **eating and/or drinking less than usual**	YES	NO
17a.6 **eating and/or drinking more than usual**	YES	NO
17a.7 **other** (please explain)	YES	NO

The following questions are about fluoride supplements that your child took:

18. Did your child take any prescription fluoride drops, tablets or vitamins with fluoride <u>during the last 4 months</u>?

1 yes
2 no **[SKIP TO QUESTION # 26 ON PAGE 9]**

19. Which fluoride supplement did your child take <u>during the last 4 months</u>?

Luride®	01	Luride® Drops
	02	Luride® 0.25 mg Lozi-Tabs
	03	Luride® 0.50 mg Lozi-Tabs
	04	Luride® 1.00 mg Lozi-Tabs
	05	Luride-SF® 1.0 mg Lozi-Tabs
Pediaflor®	10	Pediaflor® Drops
Tri-Vi-Flor®	20	Tri-Vi-Flor® 0.25 mg Drops (with or without iron)
	21	Tri-Vi-Flor® 0.50 mg Drops
	22	Tri-Vi-Flor® 1.00 mg Drops
	23	Tri-Vi-Flor® 1.00 mg Tablets
Poly-Vi-Flor®	30	Poly-Vi-Flor® 0.25 mg Drops (with or without iron)
	31	Poly-Vi-Flor® 0.50 mg Drops (with or without iron)
	32	Poly-Vi-Flor® 0.25 mg Tablets (with or without iron)
	33	Poly-Vi-Flor® 0.50 mg Tablets (with or without iron)
	34	Poly-Vi-Flor® 1.00 mg Tablets (with or without iron)
Vi-Daylin/F®	40	Vi-Daylin/F® Drops (with or without iron)
	41	Vi-Daylin/F® ADC Drops (with or without iron)
	42	Vi-Daylin/F® Chewable Tablets (with or without iron)
Sodium Fluoride	60	Sodium Fluoride (NaF) 0.50 mg Tablets
	61	Sodium Fluoride (NaF) 1.00 mg Tablets
	62	Sodium Fluoride Drops
Fluoritab®	70	Fluoritab® 1.00 mg Tablets
	71	Fluoritab® 0.25 mg Drops
	73	Fluoritab® 0.50 mg Tablets
Other	50	Other (please explain)__________________________

20. Who recommended that your child needed fluoride supplements?

1 general dentist	4 pediatrician
2 pediatric dentist	5 this research study (Iowa Fluoride Study)
3 family physician	6 other (please explain)

21. Who prescribed these fluoride supplements?

1 general dentist	4 pediatrician
2 pediatric dentist	6 other (please explain)
3 family physician	

22. During how many weeks <u>in the last 4 months</u> (17 weeks) did your child take the fluoride supplement?

|___|___| weeks

23. During the weeks your child took the fluoride supplement, how often did he or she **usually** take them?

1 daily (with only a few misses)
2 5-6 times per week
3 3-4 times per week
4 1-2 times per week
5 less than once per week

24. On the days your child took the fluoride supplement, how much did your child take?

01 1 drop daily
02 2 drops daily
03 0.25 ml daily
04 0.50 ml daily
11 0.75 ml daily
05 1 ml or 1 cc daily
06 ½ dropper full daily
07 1 dropper full daily
08 ½ tablet daily
09 1 tablet daily
10 other (please explain)

The following questions are about objects your child sucked on:

26. Did your child suck on any objects during the last 4 months ?

1 yes
2 no **[SKIP TO QUESTION # 28]**

27. Complete the table for each object your child **sucked on during the last 4 months**. Record the number of times each day they sucked on the object; how long on average they sucked on the object, each time; and whether the sucking intensity was such that you heard the sucking.

OBJECTS	NUMBER OF TIMES EACH DAY	NUMBER OF MINUTES EACH TIME	WAS SUCKING HEARD? YES 1	NO 2
27.1 thumb	|__|__|	|__|__|__|	YES	NO
27.2 other fingers, fist, hand	|__|__|	|__|__|__|	YES	NO
27.3 pacifier	|__|__|	|__|__|__|	YES	NO
27.4 toys	|__|__|	|__|__|__|	YES	NO
27.5 blanket, clothes, towel, cloth diaper	|__|__|	|__|__|__|	YES	NO
27.7 other	|__|__|	|__|__|__|	YES	NO

The following questions are about antibiotics used by your child:

28. Has your child taken any antibiotics during the last 4 months?

1 yes
2 no **[SKIP TO QUESTION # 31b]**

29. How many days during the last 4 months, did your child **actually take** antibiotics?

1 1-7 days
2 8-14 days
3 15-21 days
4 22-28 days
5 29-60 days (1-2 months)
6 61 days or more (more than 2 months)

30. Complete the table below for each antibiotic your child took. Please **circle** the type of antibiotic used and **check** either oral (swallowed by mouth), topical (applied on skin or mouth) or by intravenous (IV)

ANTIBIOTIC	**ORAL 1**	**TOPICAL 2**	**IV 3**
1. Amoxicillin (Amoxil®, Polymox®, Trimox®, etc.)			
2. Penicillin			
3. Sulfa (Septra®, Bactrim®, Gantrisin®, SMZ-TMP®, Co-trimazole®, Sulfamethoxazole, etc.)			
4. Erythromycin (EES®, Ilosone®, etc.)			
5. Cephalosporins (Ceclor®, Suprax®, Duricef®, Keflex®, Cefozil®, etc.)			
6. Pediazole®			
7. Augmentin®			
8. Nystatin® (Nilstat®, Mycostatin®, etc.)			
9. Other (please explain):			
10. Don't know name of antibiotic			

31. For what illnesses and how many episodes did your child take antibiotics during the last 4 months? Record the number of episodes, for example, 03 ear infections and 01 pneumonia.

ILLNESSES	NUMBER OF EPISODES
31.1 ear infection (otitis media)	I__I__I
31.2 pneumonia / bronchitis / RSV	I__I__I
31.3 strep throat / throat infection / pharyngitis	I__I__I
31.4 bladder / kidney / urinary infection	I__I__I
31.5 skin infection / rash / thrush	I__I__I
31.7 colds and coughs / fever / sinusitis / influenza	I__I__I
31.9 pink eye / eye infection	I__I__I
31.6 other (please explain)	I__I__I
31.8 don't know	I__I__I

31b. Has your child taken any other medications besides antibiotics during the last 4 months? (For example: Tylenol®; over the counter cold remedies and cough syrups; prescription drug such as steroids, etc.)

1 yes
2 no **[SKIP TO QUESTION # 32]**

31c. How many days during the last 4 months, did your child actually take one or more of the medications?

1 1-7 days
2 8-14 days
3 15-21 days
4 22-28 days
5 29-60 days (1-2 months)
6 61 days or more (more than 2 months)

31d. Please **write down** the names of the medications that your child took and **check** either oral (swallowed by mouth), topical (applied on skin or mouth), by intravenous (IV) or inhaled.

OTHER MEDICATIONS	ORAL 1	TOPICAL 2	IV 3	INHALER 4	SUPPOSITORY 5

The following questions are about smoking:

32. During the last 4 months, did you or anyone else smoke in your home on a regular basis?

1 yes **[Record below the number of cigarettes smoked by each person per day]**
2 no **[SKIP TO QUESTION # 33]**

SMOKING	cigarettes/day
32a. you (mother)	I___I___I___I
32b. husband or partner	I___I___I___I
32c. other:	I___I___I___I
32d. other:	I___I___I___I

33. Did any members of your household, including yourself, change their smoking habits during the last 4 months?

1 yes
2 no **[SKIP TO QUESTION # 34a]**

33a Who changed?

33b What was the change?

33c What were the reasons for the change?

The following questions are about toothbrushing:

34a. How many teeth does your child have? I___I___I teeth

36. When your child's teeth were brushed, which type of toothbrush was **usually** used during the last 4 months?

1 adult size
2 child size
3 never brushed **[SKIP TO QUESTION # 45]**

37. How often were your child's teeth **usually** brushed during the last 4 months?

1 more than three times per day
2 three times per day
3 twice per day
4 once per day
5 less than once per day
8 don't know

38. Who **usually** brushed your child's teeth during the last 4 months?

1 mother	6 mother and/or father and child
2 father	7 sibling and child
3 child (themself)	8 mother and father
4 brother or sister	5 other (please explain)

39. Was toothpaste **usually** used when your child's teeth were brushed during the last 4 months?

1 yes, regularly
2 yes, occasionally
3 no **[SKIP TO QUESTION # 45 ON PAGE 16]**

40. Who **usually** put the toothpaste on the toothbrush for your child during the last 4 months?

1 mother	6 mother and/or father and child
2 father	7 sibling and child
3 child (themself)	8 mother and father
4 brother or sister	5 other (please explain)

41. For how many minutes were your child's teeth **usually** brushed **each time** your child's teeth were brushed?

|___|___|.|___| minutes **Examples:** 15 seconds= 00.3 ½ minute= 00.5
1 minute= 01.0 10 minutes= 10.0

42. What brand of toothpaste did your child **usually** use during the last 4 months? Choose the brand name and number from the list provided. If your toothpaste is not listed, then just write the name of the toothpaste. (Use the second line if your child uses 2 brands equally).

CODE NUMBER **TOOTHPASTE NAME**

|__|__|__|____

|__|__|__|____

Children's Toothpaste

380 Baby Orajel®
Child Brite®
001 Colgate Junior®
002 Crest Sparkle®
003 Kids Aquafresh® (Bubble Gum)
004 Muppets/Oral B® (Bubble Gum)
005 Sesame St./Oral B® (Fruity Gel)

Colgate® Toothpaste

100 Colgate® Regular Paste
101 Colgate® Tartar Control Paste
102 Colgate® Baking Soda Paste
103 Colgate® Regular Gel
104 Colgate® Tartar Control Gel
105 Colgate® Baking Soda Gel
106 Colgate® Winterfresh Gel
107 Colgate® Mint Gel

Crest® Toothpaste

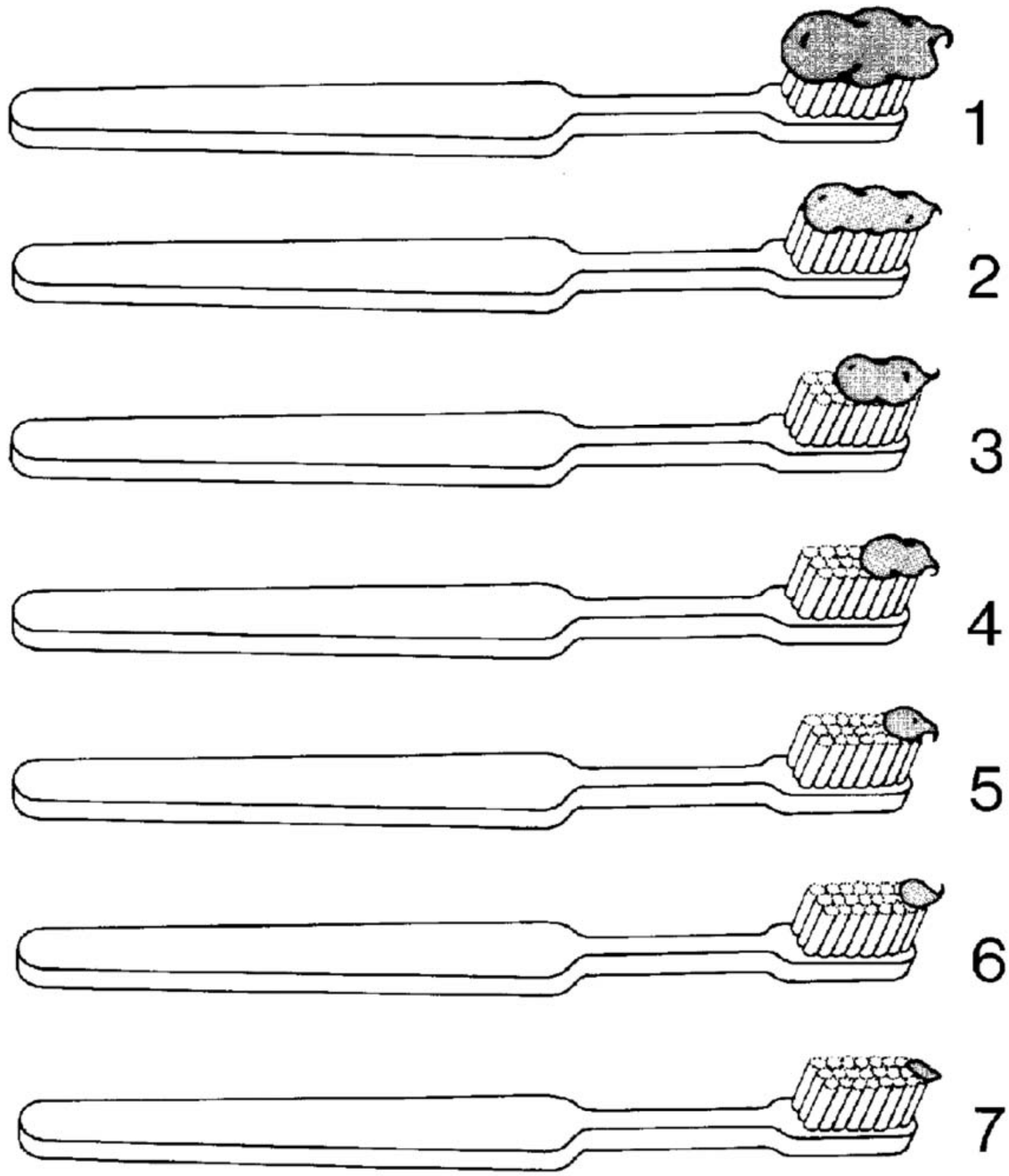
200 Crest® Regular Paste
201 Crest® Mint Paste
202 Crest® Tartar Control Paste
203 Crest® Mint Tartar Control Paste
204 Crest® Smooth Mint Tartar Control Paste
205 Crest® Regular Gel Original
206 Crest® Mint Gel
207 Crest® Tartar Control Gel
208 Crest® Mint Tartar Control Gel
209 Crest® Smooth Mint Tartar Control Gel
290 Crest® Mint Baking Soda Gel
291 Crest® Baking Soda Paste

Other Toothpaste

315 Aldi® Generic Toothpaste
320 Aim® Regular Gel
330 Aim® Tartar Control Paste
340 Aim® Tartar Control Gel
350 Extra Strength Aim® Gel
360 Amway®
361 Glister Amway®
300 Aquafresh® Paste
301 Aquafresh® Gel
310 Aquafresh® Tartar Control Paste
311 Aquafresh® Triple Protection
370 Arm & Hammer®
371 Arm & Hammer® Gel with Fluoride
375 Arm & Hammer® Baking Soda Mint
376 Arm & Hammer® Baking Soda Tooth Powder
765 Caffree®
400 Checkup®
410 Close-Up® Non Tartar Control
411 Close-Up® Gel
410 Close-Up® Tartar Control Gel
420 Dental Care® Baking Soda Mint Gel
550 Fluoride Brand K-Mart®
428 Generic Tartar Control
430 Gleem®
500 Hy-Vee® Fresh Mint Paste
510 Hy-Vee® Blue Mint Gel
520 Hy-Vee® Tartar Control
530 Hy-Vee® Tartar Control Gel
540 MacLeans®
760 Mellalevca®
730 Mentadent®
800 Osco® Tartar Control Gel
560 Oxifresh®
600 Pearl Drops®
610 Pepsodent®
612 Pepsodent® Baking Soda
778 Plus White®
770 Promise® Paste for Sensitive Teeth
810 Rembrant®
620 Sensodyne®
630 Sensodyne® Mint
650 Smokers® Toothpaste
700 Tom's®
710 Topol® Spearmint
720 Ultrabrite®
750 Viadent®
775 Zack®

43. When brushing with toothpaste, which picture best matches the amount of toothpaste **usually** placed on your child's toothbrush?

|_____| **[RECORD NUMBER 1-7 FROM TOOTHPASTE PICTURE HERE]**



44. When your child's teeth were brushed <u>during the last 4 months</u>, about how much of the toothpaste was **<u>usually</u>** swallowed?

1 all
2 almost all
3 three-quarters
4 one-half
5 one-quarter
6 very small amount
7 none at all
8 don't know

The following questions are about mouthrinse:

45. Has your child used a fluoride mouthrinse/gel at home (such as Act®, Fluorigard®, Listermint®, etc.) <u>during the last 4 months</u>?

1 yes
2 no **[SKIP TO QUESTION # 46]**

45a. Which fluoride mouthrinse/gel did your child usually use?

1 Act®
2 Fluorigard®
3 Listermint®
4 other (please explain)

45b. About how much of the mouthrinse is **<u>usually</u>** swallowed?

1 almost all
2 one-half
3 very small amount
4 none at all
8 don't know

46. Did your child participate in a fluoride mouthrinsing program at child care (or preschool) <u>during the last 4 months</u>?

1 yes
2 no
8 don't know
7 not applicable (no child care or preschool)

The following questions are about visiting the dentist:

47. Did your child have a dental (or dental hygiene) appointment <u>during the last 4 months</u>?

1 yes
2 no

48. Did your child receive a professional (office) fluoride treatment <u>during the last 4 months</u>?

1 yes
2 no

The following questions are about other vitamins NOT containing fluoride:

49. Did your child take any vitamins, other than prescription vitamins with fluoride, during the last 4 months?

    1 yes
    2 no **[GO TO THE BOTTOM OF THE PAGE]**

Please list the complete name of the vitamin that your child had. Examples are:

-Poly-Vi-Sol® Infants' Drops
-Vi-Daylin® Plus Iron Liquid
-Vi-Daylin® ADC Vitamins + Iron Drops
-Bugs Bunny® Vitamins and Minerals Chewable Tabs

Name of Vitamin: ____

50. During how many weeks in the last 4 months (17 weeks) did your child take the vitamin?

    |____|____| weeks

51. During the weeks your child took the vitamin, how often did he or she **usually** take them?

    1 daily (with only a few misses)
    2 5-6 times per week
    3 3-4 times per week
    4 1-2 times per week
    5 less than once per week

52. How much did your child take?

    01 1 drop daily
    02 2 drops daily
    03 0.25 ml daily
    04 0.50 ml daily
    11 0.75 ml daily
    05 1 ml or cc daily
    06 ½ dropper full daily
    07 1 dropper full daily
    08 ½ tablet daily
    09 1 tablet daily
    10 other (please explain)

Please check to make sure that you have completed the following:

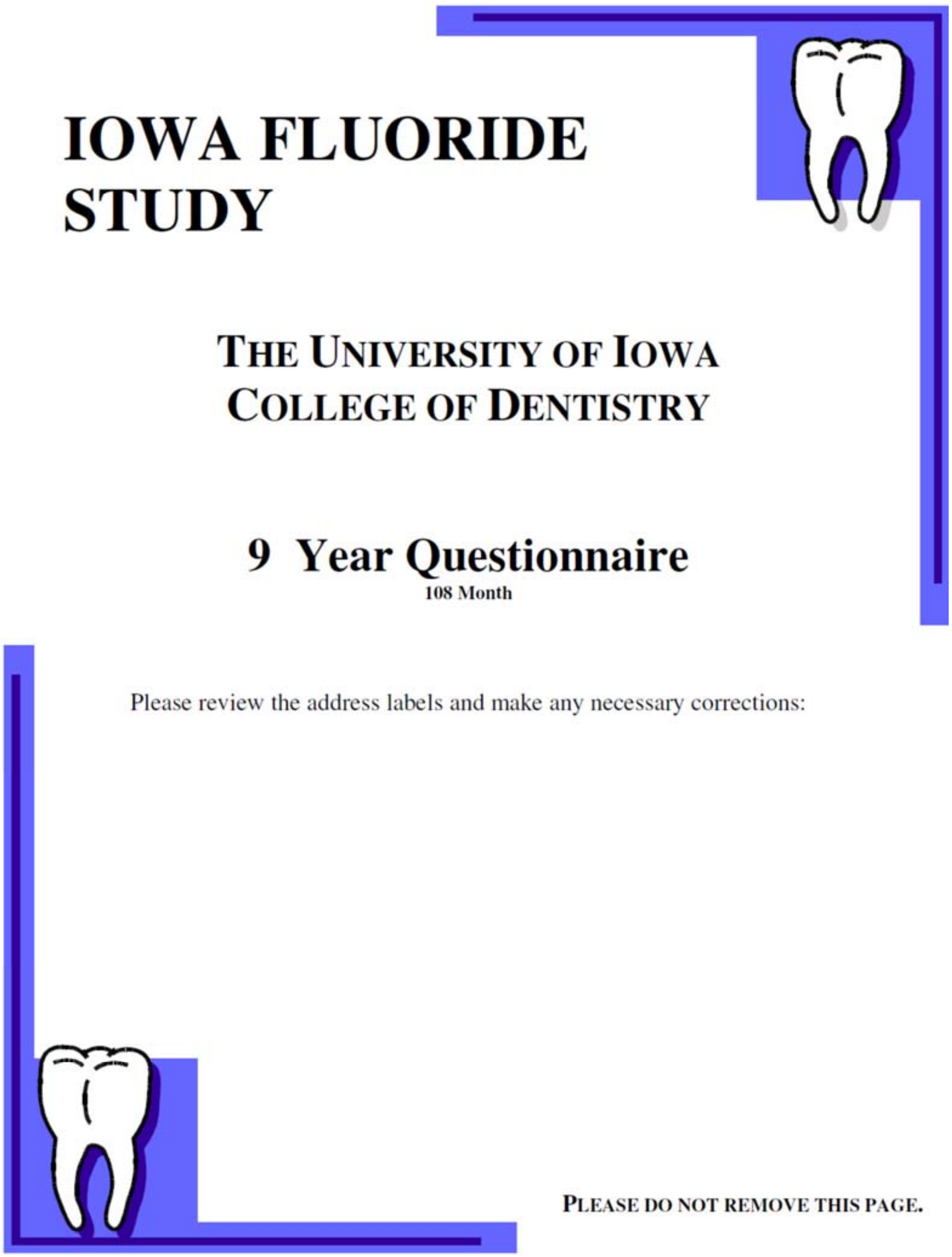
1. Today's date.. **QUESTION # 1**
2. Your child's weight... **QUESTION # 2**
3. Brand and model of water filtration at home (if applicable)...... **QUESTION # 6a**
4. Brand name of bottled water (if applicable).............................. **QUESTION # 7b**
5. Type of water at child care (if applicable)................................. **QUESTIONS 16.1 to 16.3a**
6. **The 3 day diary**

THANK YOU VERY MUCH FOR YOUR HELP!

APPENDIX C

IOWA FLUORIDE STUDY QUESTIONNAIR II

For Age 9 years and older



IOWA FLUORIDE STUDY

THE UNIVERSITY OF IOWA
COLLEGE OF DENTISTRY

9 Year Questionnaire

108 Month

Please review the address labels and make any necessary corrections:

PLEASE DO NOT REMOVE THIS PAGE.

Iowa Fluoride Study
9 Year (108 Month) Questionnaire

OFFICE USE ONLY			
Subject ID:|____|____|____|____|			
Questionnaire ID: | 2 | 5 |			
Date:	|__|__| Month	|__|__| Day	**20** |__|__| Year

DIRECTIONS & EXAMPLES:

Please review the following directions. They will assist you in the completion of the questionnaire.

Examples of how to complete questions:

- When asked to circle the response that best matches your answer:

  Type of water?

  1. Well
  2. RWA*
  3. City/Public

- When asked to provide further information regarding the source where water was consumed:

  City/State:

  ____

- When asked to write your answer in the available boxes:

  Today's Date: \|___\|___\| Month \|___\|___\| Day **20** \|___\|___\| Year

Brand Names – Brand names are needed for all beverages except milk (e.g., water, juice, pop, etc).

Questions??? If you have questions, please call the Iowa Fluoride Study office or Dr. Levy toll free at 1-888-857-7038, or if you are in the local calling area, 319-335-7026. Leave a message if no one is in the office and we will return your call.

We appreciate your participation and thank you for completing this questionnaire.

Water Information:

We are interested in the sources of water your child consumed during the past 6 months so we can track fluoride and calcium intakes. On the next page, please provide us with information regarding the source, amount, and type of water consumed at each location where your child spent time regularly. Do not include any water sources where your child spent less than 2 full weeks or less than 1 day per week on a regular basis (e.g., 1-week vacation, 1-week summer camp or 1 day per month at a location). The gray boxes are for our use, so please leave them empty.

Home Water – List water source(s) where your child lives on a regular basis. If your child has more than 1 home or more than 1 type of water at home(s), please enter it in this section.

School Water – Describe/estimate water consumed at school, school-based childcare and other activities performed at school(s).

Other Water – List sources other than home or school where your child spent at least 2 full weeks on a regular basis. Or use this space for information about other sources if not enough space was provided.

Bottled Water – List only commercial bottled water. Bottled water from another residence should be listed under "Home" or "Other".

EXAMPLE:

Water Source	Water by itself, (average ounces per day when consumed)	Weeks Consumed (26 weeks maximum)	Days/week (List number of days/week when water was consumed 1-7 days)	Type of Water? (Please circle)	Softener (Please circle)	Filter Type (Please circle)
HOME City/State: ____________ Office Use				1. Well 2. RWA* 3. City/Public	1. Yes 2. No	1. None 2. R/O** 3. Char/Carb** 4. Distillation**
SCHOOL (& school activities) Name / City: ____________ Office Use						

CALENDAR:

The following calendar can be used to count back 6 months from today's date and to calculate the number of weeks at home, school, etc.

July 2003

S	M	T	W	T	F	S
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30	31		

August 2003

S	M	T	W	T	F	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30
31						

September 2003

S	M	T	W	T	F	S
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30				

October 2003

S	M	T	W	T	F	S
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30	31	

November 2003

S	M	T	W	T	F	S
						1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23	24	25	26	27	28	29
30						

December 2003

S	M	T	W	T	F	S
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30	31			

January 2004

S	M	T	W	T	F	S
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	31

February 2004

S	M	T	W	T	F	S
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29						

March 2004

S	M	T	W	T	F	S
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30	31			

April 2004

S	M	T	W	T	F	S
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	

May 2004

S	M	T	W	T	F	S
						1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23	24	25	26	27	28	29
30	31					

June 2004

S	M	T	W	T	F	S
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30			

BEGIN HERE:

1. Please write in today's date:

|__|__| |__|__| 20 |__|__|
Month Day Year

2. What is your child's weight and height?

2.1 |___|___|___| lbs. **2.2 |___| feet |___|___| inches**

[If your child's weight from a doctor's visit is not available, please use a bathroom scale]

3. If you have moved so that the address label on page 1 is not correct, please make changes on the label on page 1 and indicate in the space below the month and year of your move.

Month |__|__| Year |__|__|__|__|

WATER INFORMATION:

Water Source	Water by itself, (average ounces per day when consumed)	Weeks Consumed (26 weeks maximum)	Days/week (List number of days/week when water was consumed: 1-7 days)	Type of Water? (Please circle)	Softener (Please circle)	Filter Type (Please circle)
4.1 HOME City/State: ________ Office Use				1. Well 2. RWA* 3. City/Public	1. Yes 2. No	1. None 2. R/O** 3. Char/Carb** 4. Distillation**
4.2 HOME City/State: ________ Office Use				1. Well 2. RWA* 3. City/Public	1. Yes 2. No	1. None 2. R/O** 3. Char/Carb** 4. Distillation**
4.3 SCHOOL (& school activities) Name / City: ________ Office Use						
4.4 SCHOOL (& school activities) Name / City: ________ Office Use						
4.5 OTHER (camp, childcare, etc.) Specify: City:________ Office Use				1. Well 2. RWA* 3. City/Public	1. Yes 2. No	1. None 2. R/O** 3. Char/Carb** 4. Distillation**
4.6 OTHER (camp, childcare, etc.) Specify: City:________ Office Use				1. Well 2. RWA* 3. City/Public	1. Yes 2. No	1. None 2. R/O** 3. Char/Carb** 4. Distillation**
4.7 BOTTLED WATER Typical Brands: 1. ________ Office Use 2. ________ Office Use 3. ________ Office Use	**Total Bottled (oz/day):**	**Total Weeks Consumed:**	**Number of Days per Week:**			

*Rural Water Association (RWA), please provide name:
____

**Filtered water, please provide the brand(s) and model(s) of the filtration system:____________________
________________________________(R/O=Reverse Osmosis; Char/Carb=Charcoal Carbon)

Food and Beverage Information

5. We are interested in your child's intake of water, beverages and calcium-containing foods **during the past week.**

Using the grid below, please answer the following questions for each item listed in the left hand column:

- Did your child consume this item?
- If yes, how many servings during the **past week** did he/she consume?
- How much did he/she typically consume at each time?
- Where indicated, please list the brand, flavor or type of food/beverage consumed and size of container as purchased. (Different sizes of containers often have different levels of fluoride.)
- Note: 1 cup = 8 ounces; ½ cup = 4 ounces.

ALL WATER, BEVERAGES and FOOD CONSUMED DURING THE LAST WEEK	**YES 1**	**NO 2**	**NUMBER OF SERVINGS PER WEEK**	**AMOUNT PER SERVING**
EXAMPLE: **Water** *(by itself, not mixed with anything)*	YES	NO	|__|__|	|__|__|oz
5.1 Water *(by itself, not mixed with anything)*	YES	NO	|__|__|	|__|__|oz
5.2 Milk *(including whole, 2%, 1%, skim or nonfat, low lactose, buttermilk, Kefir, chocolate milk, cocoa, milkshake, and milk in coffee, tea and cereal)*	YES	NO	|__|__|	|__|__|oz
5.3 Ready-to-drink juice and juice drinks **Brand Flavor Container Size**	YES	NO		
1.	Office Code		|__|__|	|__|__|oz
2.	Office Code		|__|__|	|__|__|oz
3.	Office Code		|__|__|	|__|__|oz
4.	Office Code		|__|__|	|__|__|oz
5.4 Beverages reconstituted from frozen or liquid concentrate **Brand Flavor Container Size**	YES	NO		
1.	Office Code		|__|__|	|__|__|oz
2.	Office Code		|__|__|	|__|__|oz
3.	Office Code		|__|__|	|__|__|oz
4.	Office Code		|__|__|	|__|__|oz
5.5 Beverages reconstituted from powder concentrate (e.g., Kool-Aid®, Crystal Light®, hot chocolate mix, powdered sports drinks. **For coffee and tea see 5.8/5.9**)	YES	NO		
1.	Office Code		|__|__|	|__|__|oz
2.	Office Code		|__|__|	|__|__|oz

ALL WATER, BEVERAGES and FOOD CONSUMED **DURING THE LAST WEEK**	YES 1 / NO 2	NUMBER OF SERVINGS PER WEEK	AMOUNT PER SERVING
5.6 Soda pop **Brand** **Flavor** **Container Size**	YES NO		
1.	Office Code |__|__|__|__|	|____|____|	|___|____|oz
2.	Office Code |__|__|__|__|	|____|____|	|___|____|oz
3.	Office Code |__|__|__|__|	|____|____|	|___|____|oz
4.	Office Code |__|__|__|__|	|____|____|	|___|____|oz
5.7 Ready-to-drink sports drinks **Brand** **Flavor** **Container Size**	YES NO		
1.	Office Code |__|__|__|__|	|____|____|	|___|____|oz
2.	Office Code |__|__|__|__|	|____|____|	|___|____|oz
3.	Office Code |__|__|__|__|	|____|____|	|___|____|oz
4.	Office Code |__|__|__|__|	|____|____|	|___|____|oz
5.8 Coffee (Brewed or powdered) Regular or decaffeinated? (Please Circle)	YES NO	|____|____|	|___|____|oz
5.9 Tea (Brewed or powdered) Regular or decaffeinated? (Please Circle)	YES NO	|____|____|	|___|____|oz
5.10 Wine, beer, mixed drinks (Please Circle)	YES NO	|____|____|	|___|____|oz
5.11 Foods made with mostly added water *(e.g., Jello®, dry soup, ramen noodles with broth)* **Use these equivalents: 3/4c = .75, 2/3c = .67, 1/2c = .50, 1/3c = .33, 1/4c = .25**	YES NO		**Note Decimals** ↓
EXAMPLE:		|____|____|	|___|.|___|___| cups
1.	Office Code |__|__|__|__|	|____|____|	|___|.|___|___| cups
2.	Office Code |__|__|__|__|	|____|____|	|___|.|___|___| cups
3.	Office Code |__|__|__|__|	|____|____|	|___|.|___|___| cups
5.12 Foods made with half added water *(e.g., canned soup)*	YES NO		
1.	Office Code |__|__|__|__|	|____|____|	|___|.|___|___| cups
2.	Office Code |__|__|__|__|	|____|____|	|___|.|___|___| cups
3.	Office Code |__|__|__|__|	|____|____|	|___|.|___|___| cups

ALL WATER, BEVERAGES and FOOD CONSUMED **DURING THE LAST WEEK**	YES 1	NO 2	NUMBER OF SERVINGS PER WEEK	AMOUNT PER SERVING
5.13 Foods which absorb cooking water *(e.g., rice, pasta, hot cereal, instant mashed potatoes, drained cooked ramen noodles)*	YES	NO		**Note Decimals** ↓
1.	Office Code |__|__|__|__|		|___|___|	|___|.|___|___| cups
2.	Office Code |__|__|__|__|		|___|___|	|___|.|___|___| cups
3.	Office Code |__|__|__|__|		|___|___|	|___|.|___|___| cups
4.	Office Code |__|__|__|__|		|___|___|	|___|.|___|___| cups
5.14 Yogurt: Plain, flavored, fruit, or "drinkable" varieties	YES	NO	|___|___|	|___|.|___|___| cups
5.15 Meal replacement beverages/bars: Instant Breakfast®, Ensure®, Sustacal®, Slimfast®, Nestle Sweet Success®,Sports or Energy Bar, or Slimfast® Bar	YES	NO	|___|___|	|___|.|___|___| cups or bars
5.16 Frozen milk-based desserts: Regular or soft serve ice creams, ice milk, frozen yogurt, ice cream bar or sandwich	YES	NO	|___|___|	|___|.|___|___| cups or bars
5.17 Milk-based desserts: Pudding, bread pudding, custard, flan, rice and tapioca pudding, cream pie	YES	NO	|___|___|	|___|.|___|___| cups
5.18 Cottage cheese	YES	NO	|___|___|	|___|.|___|___| cups

For the following food items, how many medium servings did your child consume during the last week?

EXAMPLE: Cheese, cheese sauce: Sandwiches with cheese, cheese & crackers, nachos, cheese or cheese sauce on baked potato, salad, or vegetables	YES	NO	|___|___| Servings Per Week	
5.19 Cheese, cheese sauce: Sandwiches with cheese, cheese & crackers, nachos, cheese or cheese sauce on baked potato, salad, or vegetables	YES	NO	|___|___| Servings Per Week	
5.20 Cheese in mixtures: Macaroni & cheese, lasagna, pizza, cheese soup, cheese nuggets, cheesecake, eggs with cheese	YES	NO	|___|___| Servings Per Week	
5.21 Green leafy vegetables: Cooked dark green vegetables like spinach, broccoli, cabbage, brussels sprouts, cabbage slaw	YES	NO	|___|___| Servings Per Week	
5.22 Dry Beans: Pork & beans, baked beans, refried beans, bean soup, garbanzos (don't count if only sprinkled on salad)	YES	NO	|___|___| Servings Per Week	

Fluoride Supplements Information

6. Did your child take any prescription fluoride drops, tablets or vitamins with fluoride <u>during the last 6 months</u>?

1. yes
2. no **[SKIP TO QUESTION #11]**

7. Circle the number next to the fluoride supplement(s) your child took <u>during the last 6 months</u>:

01 Luride® Drops
02 Luride® 0.25 mg Lozi-Tabs
03 Luride® 0.50 mg Lozi-Tabs
04 Luride® 1.00 mg Lozi-Tabs
05 Luride-SF® 1.0 mg Lozi-Tabs
10 Pediaflor® Drops
32 Poly-Vi-Flor® 0.25 mg Tablets (with or without iron)
33 Poly-Vi-Flor® 0.50 mg Tablets (with or without iron)
34 Poly-Vi-Flor® 1.00 mg Tablets (with or without iron)
60 Sodium Fluoride (NaF) 0.50 mg Tablets
61 Sodium Fluoride (NaF) 1.00 mg Tablets
70 Fluoritab® 1.00 mg Tablets
73 Fluoritab® 0.50 mg Tablets

50 Other:
(Please explain: Brand/Dosage/Type/Frequency) ____________________

8. How many weeks <u>in the last 6 months</u> (26 weeks) did your child take the fluoride supplement?

|___|___| **weeks**

9. During the weeks your child took the fluoride supplement, how often did he or she <u>usually</u> take them?

1. daily (with only a few misses)
2. 5-6 times per week
3. 3-4 times per week
4. 1-2 times per week
5. less than once per week

10. On the days your child took the fluoride supplement, how much did your child take?

8. ½ tablet or lozenge
9. 1 tablet or lozenge
10. other (please explain)________________

Toothbrushing Information

11. When your child brushed his/her teeth, what type of toothbrush was usually used during the last 6 months?

1. adult size
2. child size
5. electric
3. never brushed in the last six months **[SKIP TO QUESTION #18]**

12. How often did your child brush his/her teeth during the last 6 months?

1. more than three times per day
2. three times per day
3. twice per day
4. once per day
5. less than once per day
8. don't know

13. Was toothpaste usually used when your child brushed his/her teeth during the last 6 months?

1. yes, regularly
2. yes, occasionally
3. no **[SKIP TO QUESTION #18]**

14. Each time your child brushed his/her teeth, how many minutes did your child usually brush?

|___|___|.|___| minutes

Examples: 15 seconds = 00.3 ½ minute = 00.5
45 seconds = 00.8 1 minute = 01.0

15. What brand of toothpaste did your child usually use during the last 6 months? Write the complete brand name of toothpaste, flavor, paste or gel, etc. (Use the second line if your child uses 2 brands equally.)

TOOTHPASTE NAMES

____

____

OFFICE USE ONLY
Code Number

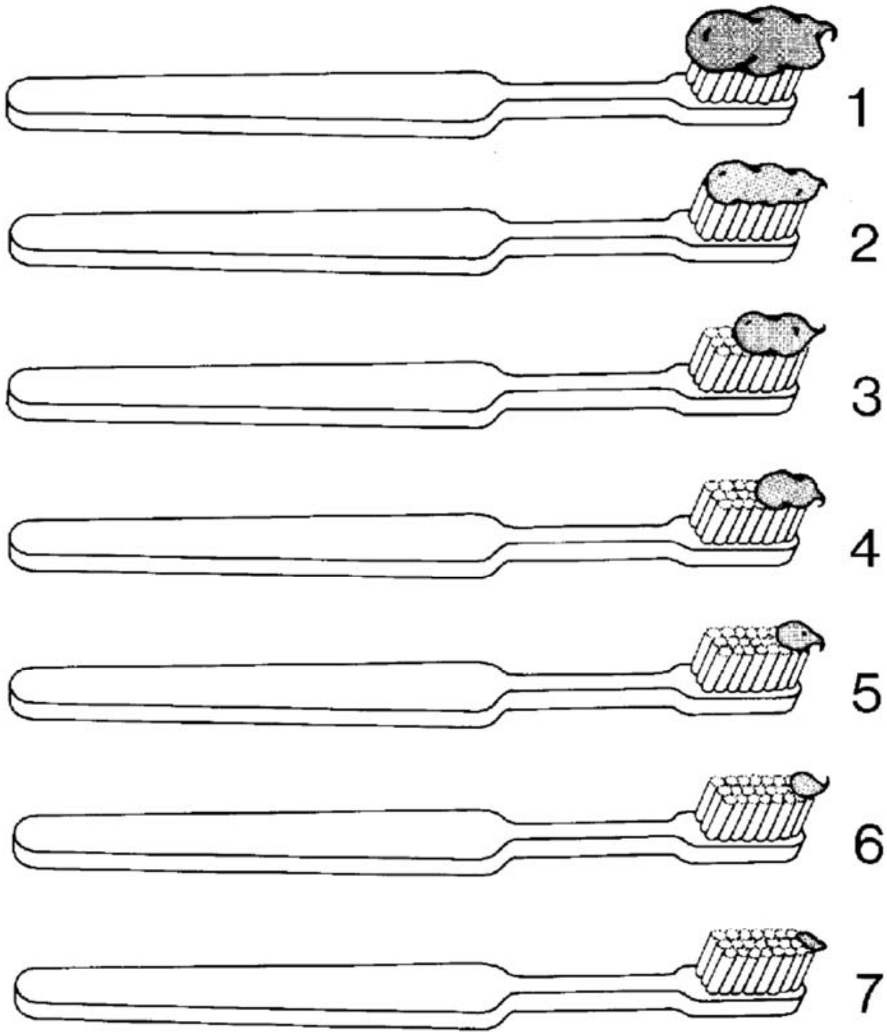
|___|___|___|

|___|___|___|

For example: Sesame Street/Oral B® Fruit Gel, Kids Aquafresh® Bubblegum, Muppets/Oral B® Bubblegum, Colgate® Baking Soda Paste, Colgate® Tartar Control Gel, Crest® Regular Paste, Crest® Mint Tartar Control Paste.

16. **When brushing with toothpaste, which picture best matches the amount of toothpaste your child usually placed on the toothbrush?**

|_____| [Record Number 1-7 from picture below]



17. **When your child brushed his/her teeth during the last 6 months, about how much of the toothpaste was usually swallowed?**

1. All
2. Almost all
3. Three-quarters
4. One-half
5. One-quarter
6. Very small amount
7. None at all
8. Don't know

Fluoride Mouthrinse and Gel Information

18. Did your child use a *FLUORIDE* mouthrinse or gel at home (such as Act®, Fluorigard®, etc.) during the last 6 months?

1. yes
2. no **[SKIP TO QUESTION #19]**

18a. Which *FLUORIDE* mouthrinse or gel did your child usually use?

1. Act®
2. Fluorigard®

10. Other (Please explain Brand/Dosage/Type/Frequency)____

18b.

OFFICE USE ONLY
Code Number
|__|

18c. About how much of the *FLUORIDE* mouthrinse or gel was usually swallowed?

1. almost all
2. one-half
3. very small amount
4. none at all

8. don't know

19. Did your child participate in a *FLUORIDE* mouthrinsing program at child care or school during the last 6 months?

1. yes
2. no

8. don't know

Dentist Information

20. Did your child have a dental (or dental hygiene) appointment during the last 6 months?

1. yes
2. no

21. Did your child receive a professional (office) fluoride treatment during the last 6 months?

1. yes
2. no

Sucking Information

22. Did your child suck on any objects during the last 6 months ?

1. yes
2. no [SKIP TO QUESTION #24]

23. Complete the table for each object your child sucked on during the last 6 months. Record the approximate number of minutes each day they sucked on the object.

OBJECTS	APPROXIMATE NUMBER OF MINUTES EACH DAY
23.1 thumb	|__|__|
23.2 other fingers, fist, hand	|__|__|
23.7 other	|__|__|

Supplements (Including Vitamins, Minerals & Multivitamins) Without Fluoride Information

24. Did your child take any supplements other than prescription vitamins with fluoride during the last six months?

1. yes
2. no [SKIP TO QUESTION #28]

24a. Please list the complete name of the supplement(s) that your child took.

NOTE: For any Vitamin C, Vitamin D, Calcium, or Iron supplement, please list the tablet size. *(Example: Spring Valley® Vitamin C 500 mg Tablets)*

BRAND NAME:	TABLET SIZE:	OFFICE USE ONLY Code Number
Supplement 1.__________		|__|__|__|
Supplement 2.__________		|__|__|__|
Supplement 3.__________		|__|__|__|

MULTIVITAMIN EXAMPLES:
Bugs Bunny® Children's Chewable Tablets
Flintstones® with Extra C Children's Chewable Tablets
Spring Valley® (Walmart)Children's Chewable Complete
Centrum Jr.® plus Iron Tablets

OTHER VITAMIN & MINERAL EXAMPLES:
Tums® Regular Strength 200 mg
Tums® Ultra Strength 800 mg
Spring Valley® Vitamin C Tablets 500 mg
Viactiv® Calcium Chews 500 mg
Oscal® 250 plus D Tablets

25. How many weeks <u>in the last 6 months</u> (26 weeks) did your child take the supplement(s)?

25.1 supplement 1: |____|____| weeks

25.2 supplement 2: |____|____| weeks

25.3 supplement 3: |____|____| weeks

26. During the weeks your child took the supplement(s), how often did he or she <u>usually</u> take them?

26.1 Supplement 1:

1. daily (with only a few misses)
2. 5-6 times per week
3. 3-4 times per week
4. 1-2 times per week
5. less than once per week

26.2 Supplement 2:

1. daily (with only a few misses)
2. 5-6 times per week
3. 3-4 times per week
4. 1-2 times per week
5. less than once per week

26.3 Supplement 3:

1. daily (with only a few misses)
2. 5-6 times per week
3. 3-4 times per week
4. 1-2 times per week
5. less than once per week

27. On the days your child took the supplement(s), how much did your child take?

27.1 Supplement 1:

8. ½ tablet
9. 1 tablet
10. other (please explain): ______________________________

27.2 Supplement 2:

8. ½ tablet
9. 1 tablet
10. other (please explain): ______________________________

27.3 Supplement 3:

8. ½ tablet
9. 1 tablet
10. other (please explain): ______________________________

Fortified Foods Information

Food manufacturers often add calcium to foods that may or may not naturally contain calcium. These foods are usually labeled "with added calcium" or "calcium fortified". Foods most commonly fortified with calcium include breakfast cereals, juices, and breads.

28. During the last 6 months, did your child consume any calcium-fortified foods or beverages?

1. yes
2. no **[End of Questionnaire Thank You!]**

29. Please list the products (include brand names) your child consumed, the average number of servings consumed per week, and the serving size.

Product/Brand Name:	Number of Servings per Week:	Serving Size (include units)	Office Use Only Code
Example ______________________	_____/ Week	____________	| | | | | |
Example ______________________	_____/ Week	____________	| | | | | |
Example ______________________	_____/ Week	____________	| | | | | |
1.______________________	_____/ Week	____________	| | | | | |
2.______________________	_____ / Week	____________	| | | | | |
3.______________________	_____ / Week	____________	| | | | | |
4.______________________	_____ / Week	____________	| | | | | |
5.______________________	_____ / Week	____________	| | | | | |
6.______________________	_____ / Week	____________	| | | | | |
7.______________________	_____ / Week	____________	| | | | | |

Thank you very much for your help!

APPENDIX D

DIETARY DIARY

Day:__________________ Date:________/__________/________ Was this a child care day? __________Yes _________No

Was your child ill today? __________Yes _________No

Please remember to write down everything that your child eats and drinks

Brand names are needed for all beverages. We will determine the fluoride content from the specific brand name listed

If you have any questions, please refer to the example pages or call us **collect** at 319-335-7026 or 319-335-7182.

Time am/pm	**Place:** H-Home C-Child care O-Out	**Food and Beverages**	**Brand Name**	**Type and/or Preparation:** ready-to-eat; frozen, liquid or powder concentrate)	**Water from:** H-Home tap C-Child care O-Out B-Bottled	**Amount Ate and Drank**	**DO NOT WRITE HERE**

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