PERGAMON

Archives of Oral Biology 47 (2002) 491–498

Archives of Oral Biology

www.elsevier.com/locate/archoralbio

Relationships between dietary behaviours, oral hygiene and mutans streptococci in dental plaque of a group of infants in southern England

Mina Habibian [a], David Beighton [b], Rebecca Stevenson [c], Margaret Lawson [d], Graham Roberts [e,*]

[a] *Health Promotion, Disease Prevention and Epidemiology, University of Southern California, School of Dentistry, 925 West 34th Street, Los Angeles, CA 90089-0641, USA*

[b] *Department of Oral Microbiology, GKT Dental Institute, Caldecot Road, London SE5 9RW1, UK*

[c] *The Eastman Dental Institute for Oral Health Care Sciences, 256 Gray's Inn Road, London WC1X 8LD, UK*

[d] *The Institute of Child Health, 30 Guilford Street, University College London, London WC1N 1EH, UK*

[e] *The Eastman Dental Institute and The Institute of Child Health, University College London, 256 Gray's Inn Road, London WC1X 8LD, UK*

Accepted 3 January 2002

Abstract

This report is part of a prospective study on dietary behaviours, feeding practices, oral hygiene and dental health during the first 18 months of life in a cohort of infants living in southern England. In this part of the study the prevalence of mutans streptococci in the plaque of 1-year-old children and its correlation with sociodemographic status, dietary behaviour and oral hygiene practices over the first 12 months of life were studied. Dietary habits, oral hygiene and dental health at 18 months were also studied. The study group comprised 163 infants. At 12 months of age all children had a dental examination and a plaque sample was removed from the labial surfaces of upper incisors for microbiological examination. A further dental examination took place at 18 months of age; no plaque was sampled. Longitudinal dietary information was obtained with a 3-day food diary at 6, 12 and 18 months of age. Parents/carers completed two structured questionnaires on sociodemographic factors, oral hygiene and feeding behaviour over the first 18 months of life when their children were 12- and 18-month-old. No child exhibited dental caries at 12 and 18 months of age; 18 and 25% had visible dental plaque at 12 and 18 months, respectively. Seven children (4%) had detectable mutans streptococci in their plaque at 12 months of age. Children who had started brushing their teeth or who had had their teeth brushed by 12 months of age were less likely to have detectable mutans streptococci in their plaque than those who had not ($P = 0.02$). The amount of mutans streptococci was significantly correlated with the total number of eating/drinking events per day ($P < 0.001$) and bordered on significant correlation with the mean daily frequency of consumption of foods and drinks containing non-milk extrinsic sugars ($P = 0.05$). None of the sociodemographic variables—sex, social class, mother's level of education—were associated with the detection of mutans streptococci in this group of infants. Multiple logistic regression analysis revealed that two variables were independently and positively correlated with the presence of mutans streptococci in the plaque; these were age when tooth brushing started and total eating and drinking events per day.

Keywords: Dental caries; Mutans streptococci; Dental plaque; Infants; Oral hygiene; Diet; Sugars

1. Introduction

Early childhood caries or nursing caries are the terms describing a pattern of dental caries, observed in late infancy and early childhood (Ripa, 1978; Tinanoff and O'Sullivan, 1997), which affects the labial, mesial, distal and palatal

Abbreviation: cfu, colony-forming unit

* Corresponding author. Tel.: +44-207-915-1022; fax: +44-207-915-2329.

E-mail address: g.roberts@eastman.ucl.ac.uk (G. Roberts).

0003-9969/02/$ – see front matter
PII: S0003-9969(02)00017-1

surfaces of primary maxillary incisors. Several studies have shown wide variations in the prevalence of early childhood caries, from 0.5% to as high as 80% in different countries, populations and age groups (Ripa, 1988; Wendt et al., 1991).

Mutans streptococci (*S. mutans* and *S. sobrinus)* are considered causative agents of dental caries (Hamada and Slade, 1980). In early childhood caries, mutans streptococci are the dominant caries-associated microorganisms (van Houte et al., 1982). The tooth surface is the major habitat of mutans streptococci in the mouth (Krasse et al., 1967; Carlsson et al., 1970). The hard, non-shedding surfaces and retention areas of the dentition are unique environments for bacterial attachment and proliferation. Mutans streptococci are rarely isolated from the mouth before tooth eruption (Caufield et al., 1993); as the number of erupted teeth increases there is an increase in the frequency of their isolation (Catalanotto et al., 1975; Fujiwara et al., 1991).

Poor oral hygiene is one of the risk factors for dental caries (Sclavos et al., 1988; Eronat and Eden, 1992; Muller, 1996). Children with poor oral hygiene have more decayed teeth (Kleemola-Kujala, 1978; Kalsbeek and Verrips, 1994).

Tooth surfaces most likely to become carious often exhibit visible plaque, in which mutans streptococci may constitute a high proportion of the total cultivable flora before or during caries initiation (van Houte, 1994). A cariogenic microflora that can initiate dental caries may be established at a very early age (Carlsson et al., 1975; Grindefjord et al., 1991; Roeters et al., 1995).

Oral microorganisms, especially *S. mutans*, utilise dietary sucrose to form a sticky plaque matrix that enables them to adhere to the tooth. Sugar restriction has been shown to diminish the occurrence of *S. mutans* in the mouth (Wennerholm et al., 1995a). Dietary carbohydrates serve as substrate in the production of organic acids that demineralise the teeth (Kleinberg, 1970; Loesche, 1986).

Early colonisation with mutans streptococci has been associated with higher caries prevalence in the deciduous dentition (Köhler et al., 1988). The influence of factors affecting the colonisation of mutans streptococci in 1-year-old children has been studied; the results indicate that maternally influenced behaviours, such as dietary habits that may predispose to early colonisation, are already established at 1 year (Grindefjord et al., 1991). Nonetheless, studies of factors that affect early colonisation with mutans streptococci in infants are limited, which may be due to the considerable difficulties inherent in undertaking them. Our main aim now was to investigate in a prospective study the prevalence of mutans streptococci in plaque and the association between this plaque, oral hygiene, dietary habits and sociodemographic factors in a group of infants.

2. Materials and methods

This report is part of a prospective study on the role of diet, feeding and oral hygiene behaviour in the dental health of infants and toddlers (Habibian et al., 2001). Families of all ($n = 2300$) new-borns between April 1995 and May 1996 (13 months) in mid-Surrey, southern England, were invited to include their children in the study. A total of 1380 families consented for their infants to participate; 71 families refused. Lack of time, future plans to move out of the area and lack of interest were their reasons given for not participating. Two reminders were sent to non-respondents and their reasons for not participating were sought; 859 families did not reply (Table 1). Phone-call reminders were not possible, as the investigators did not have access to the families' phone numbers. The average age of children at recruitment was 3 (S.D. 1.6) months.

Ethical approval for the project was obtained from the ethical committee at Epsom NHS Trust in the county of Surrey in the UK. Dietary data were obtained at 6, 12 and 18 months of age by using a 3-day food diary. Table 1 shows the response rates to each dietary assessment. The diaries were sent to all parents or carers who were still participating in the study at the time when their children were approximately 6-month-old ($n = 1380$) and 12-month-old ($n = 1340$). For the 18-month assessment it was necessary to change the method of selection. Only parents who had already completed the first two diaries were asked to complete the 18-month diary; on this basis, 673 parents were selected for completion of that diary. Parents/carers recorded food intake, using household measures, for 3 days (2 weekdays and 1 weekend day) when their children were eating normally. Recordings began after midnight; the name, amount and time of taking any food and drink were written down. Non-compliant parents were followed up.

The families who participated were invited to have a dental examination carried out on their children at 12 months and again at 18 months of age. The examinations were done at health centre clinics in the Epsom NHS Trust and were free of charge. Two reminders were sent to non-attendants. One examiner (MH), who was calibrated against an experienced community dental officer, examined all the children. Only a mouth mirror and light were used. A tooth was regarded as erupted if any of its surfaces were present in the mouth. At 12 months of age a plaque sample was removed from the labial surfaces of central incisors with a sterile toothpick; if the central incisors were not erupted the lateral incisors were used. The toothpick was moved over the tooth surface gingivo-incisally. Plaque samples for microbial analysis were immediately placed into 1 ml of fastidious anaerobe broth (LabM, Bury, Lancs, UK) supplemented with 30% glycerol and transported to the laboratory, where they were stored at $-70\,^{\circ}C$ until required. The teeth were then cleaned and dried with a cotton-wool roll and all surfaces were examined visually for dental caries (WHO, 1987) and the presence or absence of dental plaque.

At the time of or after the dental examinations the parents completed a questionnaire containing 68 questions about sociodemographic factors, feeding practices, oral hygiene and general health.

Table 1
Response rates to recruitment and dietary assessments at 6, 12 and 18 months of age

	No. of children	Percentage
Recruitment response at 4–12 weeks of age:		
Invited	2300	
Refused	71	
No contact	859	
Consented	1380	60 (of all invited)
First diet assessment response at 6 months of age:		
Dropped out/moved away	40	
$Participant_6$ (food diary 1 sent)	1340	
Food diary 6 returned	900	67 (of all sent diary 1)
Second diet assessment response at 12 months of age:		
Dropped out/moved away/dead	32	
$Participant_{12}$ (food diary 2 sent)	1308	
Food diary 12 returned	605	46 (of all sent diary 2)
Third diet assessment response at 18 months of age:		
Dropped out/moved away	119	
$Participant_{18}$	1189	
Selected for third diet assessment	673	
Food diary 18 returned	259	38 (of all sent diary 3)
All diet assessments response at 6, 12 and 18 months of age:		
All food dairies sent	642	
All food dairies returned	207	32 (of all sent three diaries)

Table 2 shows the response rates to the dental examinations at 12 and 18 months of age. A total of 163 child/parent pairs completed three food diaries (6, 12 and 18 months), two dental examinations (12 and 18 months) and had a plaque sample removed at 12 months of age.

2.1. Microbiological methods

The plaque samples were shaken with sterile glass beads, then diluted in fastidious anaerobe broth; 100 μl of the neat and the diluted sample were spread on mitis salivarius agar (Difco) supplemented with 0.2 units/ml bacitracin and sucrose (15% w/v) (Gold et al., 1973). Inoculated plates were incubated in an anaerobic chamber for 3 days and the number of mutans streptococci counted. The detection level was 10 cfu per plaque sample.

2.2. Analysis of data

Each returned food diary was checked for completeness by a trained nutritionist. One observer (RS) calculated the frequencies of consumption of non-milk extrinsic sugars

Table 2
Response rates to dental examinations at 12 and 18 months of age

	No. of children	Percentage
Consented to take part in the study	1380	
First dental examination (12 months of age):		
Dropped out/moved away/dead	72	
$Participant_6$: (invited for exam 1)	1308	
Attended dental exam 1	955	73 (of all invited for exam1)
Second dental examination (18 months of age):		
Dropped out/moved away/dead	119	
$Participant_{12}$: (invited for exam 2)	1189	
Attended dental exam 2	640	54 (of all invited for exam 2)
Both dental examinations (12 and 18 months of age):		
Invited	1189	
Attended	572	48 (of all invited for both exams)

(episodes per day). Non-milk extrinsic sugars are all sugars not contained within the cellular structure of food, whether natural or refined, excluding lactose in milk and milk products (Department of Health and Social Security, 1989). Formula milks and soya milks containing glucose/maltidextrin were included into this category. An episode was defined as an occasion when drinks, foods or both were consumed within one of the allocated time periods, or at least a 30 min period had elapsed between the previous consumption of foods/drinks. The daily frequency of consumption of all foods/drinks, including water as a drink and breast milk, was calculated using this method.

The social class of each child was determined from the Registrar General's classification based on the fathers' occupation (Registrar General, 1993). They were classified to two non-manual and manual social classes.

All data were analysed with SPSS software (SPSS Inc., Chicago, IL, USA). The microbial counts were transformed to $\log_{10}$ (10 cfu) per sample. The data were tested for normality using a normal plot or the Shapiro–Wilk test as appropriate. The majority of data were not normally distributed; therefore non-parametric methods including Mann–Whitney and Spearman rank correlation tests were used for comparison and correlation between groups (Altman, 1991); χ^2 or Fisher's exact tests were used for the comparison of different variables between groups. The statistical level of significance was set at $P < 0.05$.

3. Results

The sample comprised 163 children, 51% male and 49% female; 84% came from a non-manual social class and the remaining 16% from a manual social class; one child (0.6%) came from a family whose father was unemployed. The mothers' level of education was derived from their highest educational qualification. Overall, 31% of mothers had only high school education; 69% had a college, university or professional degree.

Dental caries was not diagnosed in any child at either 12 or 18 months of age. Visible plaque was observed in 18 and 25% of children at 12 and 18 months of age, respectively. The mean number of teeth present in the mouth was 6.4 (S.D. 2.8) and 13.6 (S.D. 2.8) at 12 and 18 months of age, respectively.

Seven children (4%) had detectable mutans streptococci in their plaque at 1 year of age. The count ($\log_{10}$) of mutans streptococci among children with detectable levels ranged between 1.32 and 2.66 cfu per sample.

At 12 months of age, 90% of children had already started brushing their teeth or had had them brushed by their parents. Among this group the mean age when tooth brushing started was 8.1 (S.D. 2.5) months: 36% of these children had their teeth brushed or brushed them themselves more than once a day (Fig. 1); 96% of these children had received help from their parents (Fig. 2); 84% of children who had started brushing their teeth or had had them brushed by 12 months of age were using a fluoride toothpaste.

At 18 months of age, 96% of children brushed their teeth themselves or had had them brushed and 99% of them were using a fluoride toothpaste; 52% of them had their teeth brushed or brushed them themselves more than once a day compared with 36% at 12 months (Fig. 1). The number of children who brushed their teeth themselves at 18 months (7.5%) was higher than at 12 months of age (3%) (Fig. 2). Of children who had had their teeth brushed or brushed them themselves by 12 months of age, 85% came from a non-manual social class, compared with 15% who came from a manual social class ($P = 0.3$). The same proportions were observed at 18 months of age.

The mean daily frequency of eating/drinking events (including water as drink and breast feeding) was 6.9 (S.D. 1.8) times at 6 months, 6.8 (S.D. 1.7) times at 12 months and 6.8 (S.D. 1.8) times at 18 months of age. The mean frequency of consumption of foods/drinks containing non-milk extrinsic sugars was 3.1 (S.D. 1) times per day at 6 months, 4 (S.D. 1.3) times per day at 12 months and 4.5 (S.D. 1.3) times per day at 18 months of age. The mean

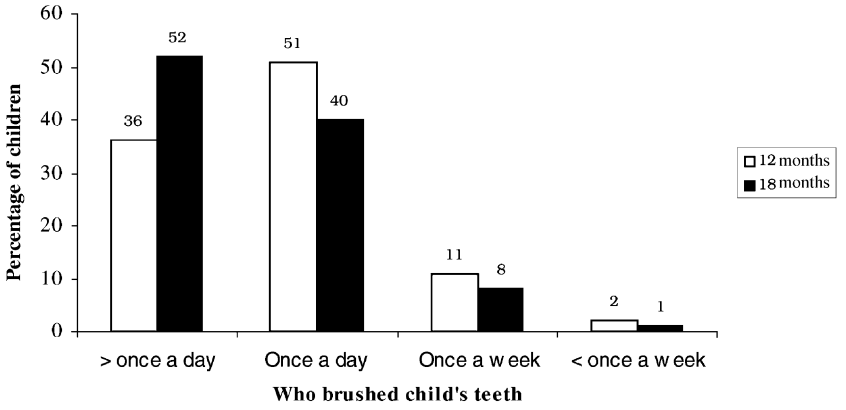

Fig. 1. Frequency of tooth brushing.

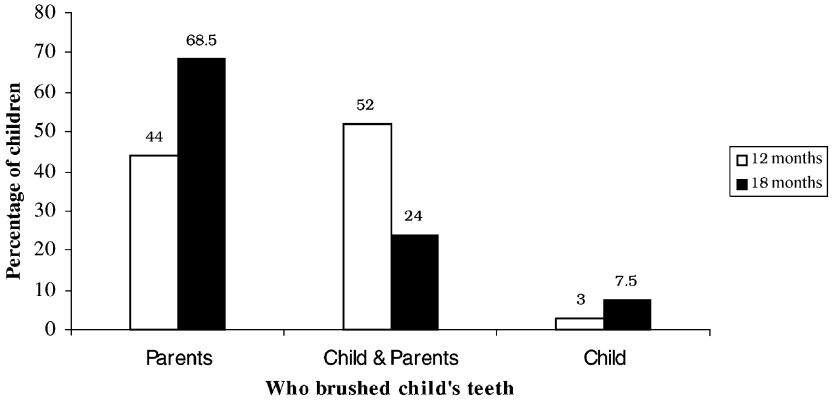

Fig. 2. Percentage of children who received assistance with tooth brushing or brushed their teeth themselves at 12 and 18 months of age.

frequency of consumption of 18 different categories of foods and drinks was also investigated. Detailed information on the children's dietary intakes and their correlation with dental health has been reported elsewhere (Habibian et al., 2001). The correlations between the detection of plaque mutans streptococci at 12 months, dietary behaviour at 6 and 12 months, oral hygiene behaviour over the first 12 months of life as well as sociodemographic factors are reported here.

Of children who had started brushing by 12 months of age, 3% had detectable mutans streptococci, compared with 19% of children who had not ($P = 0.003$) (Table 3). The frequency of tooth brushing, number of teeth and visible plaque were not significantly associated with the

Table 3
Correlations between demographic factors, oral hygiene, dietary habits and detection of mutans streptococci in the plaque at 12 months of age

Variables	Children with mutans streptococci ($n = 7$)	Children with no mutans streptococci ($n = 156$)	P
Sex:			
Girls	4	80	0.7
Boys	3	76	
Social class:			
Manual	1	25	0.9
Non-manual	6	131	
Mothers level of education:			
College, university, professional	4	106	0.7
High school only	3	50	
Visible plaque:			
Yes	1	29	0.7
No	6	127	
Brushing at 12 months:			
Yes	4	142	0.003*
No	3	13	
Mean frequency of consumption of non-milk extrinsic sugars at 12 months:			
>4 times per day	1	43	0.4
≤4 times per day	6	113	
Mean frequency of total eating/drinking events at 12 months:			
>6.8 times per day	6	56	0.008*
≤6.8 times per day	1	100	

χ^2 or Fisher's exact test were used to study the correlations.
* Significant at $P < 0.05$.

Table 4
Spearman rank correlation coefficients between dietary variables at 6 and 12 months and counts of plaque mutans streptococci at 12 and 18 months of age

Variables	r_s[a]	P
Frequency of eating/drinking at 6 months	0.09	0.2
Frequency of eating/drinking at 12 months	0.25	0.001*
Frequency of non-milk extrinsic sugars at 6 months	−0.09	0.24
Frequency of non-milk extrinsic sugars at12 months	0.15	0.05

[a] r_s, Spearman's rank correlation coefficient.
* Significant at $P = 0.05$.

detection of mutans streptococci. There was no correlation between any of the sociodemographic factors—sex, social class, mothers' level of education and detection of mutans streptococci (Table 3).

Table 4 shows the correlation between dietary variables at 6 and 12 months of age and the accumulation of mutans streptococci at 12 months of age. There were no significant correlations between the mean daily frequency of total eating/drinking events and the mean daily frequency of consumption of foods/drinks containing non-milk extrinsic sugars at 6 months and the detection of mutans streptococci at 12 months ($r_s = 0.09$, $P = 0.2$), ($r_s = -0.09$, $P = 0.2$). The mean daily frequency of total eating/drinking events at 12 months and amounts of mutans streptococci in the plaque were positively and highly significantly correlated ($r_s = 0.4$, $P < 0.001$). The correlation between mutans streptococci and the mean daily frequency of consumption of foods and drinks containing non-milk extrinsic sugars at 12 months was very low ($r_s = 0.15$) and bordered on statistical significance ($P = 0.05$). Nevertheless, the mean frequency of consumption of non-milk extrinsic sugars by children with detectable mutans streptococci was 5 (S.D. 1.5) times per day, compared with 3.9 (S.D. 1.2) times per day by children with no detectable mutans streptococci. The difference in the means between the two groups was statistically significant ($P = 0.04$).

There were no significant correlations between frequency of consumption of any of the 18 different categories of foods/drinks at both 6 and 12 months and counts of mutans streptococci.

Multiple logistic regression models were used to estimate the association between various independent variables and the presence or absence of plaque mutans streptococci. Using both forward and backward logistic regression analysis the variables shown in Table 3 were examined as indicator variables. The final model always included two variables, i.e. (brushing at 12 months) and (mean total eating/drinking at 12 months); they were both significant at $P < 0.05$. The model showed that:

(a) children who had not started brushing their teeth or had not had them brushed by 12 months of age were more likely to have detectable mutans streptococci in their plaque than those who had;
(b) children who ate/drank more than 6.7 times per day were more likely to have detectable mutans streptococci in their plaque.

4. Discussion

The sample comprised a group of children with three completed diet diaries and two dental examinations out of all those (1380) who volunteered to participate. Not all parents who agreed to their children's participation completed all elements of the study. For instance, the response rate for the dietary survey declined from 67% at 6 months to 45% and 38% at 12 months and 18 months, respectively. The response rate for the dental examination decreased from 73% at 12 months to 54% at 18 months.

The main difficulty with cohort studies is that some participants are not followed for the full length of the study because they move or lose interest (Altman, 1991). Here, the response rates for dental examinations were higher than for the diet diaries; this might reflect the parents' lack of perceived benefits in completing the diaries. The longitudinal nature of the study (three diet diaries, two dental examinations and the completion of two questionnaires over the 18-month period) may also have contributed to the low completion rate. It is not known how the children who completed all aspects of the study differed in dietary habits and dental health from those who either did not participate or did not complete all aspects of the study. Therefore, the sample group cannot be regarded as random and may not be representative of the population. Despite the non-response and low completion rates the number of children with complete data were sufficient for the study's objectives.

Children who did participate were mainly from a non-manual social class and therefore it may not be appropriate to generalise the findings to all children; any extrapolation of these results should be attempted with considerable care.

The age of initial colonisation with mutans streptococci and factors that might affect their acquisition are controversial. Although some investigators were not able to detect the organism in infants with only primary incisors (Catalanotto et al., 1975), others have shown that 6% of 1-year-old had detectable mutans streptococci (Grindefjord et al., 1991). Only 4% of children in the present study had detectable mutans streptococci in their plaque at 12 months.

Differences between reports of the acquisition of mutans streptococci in infants could reflect true differences between populations, or might be due to differences in sampling methods. In the human mouth, mutans streptococci are generally detected and enumerated from a saliva sample, dental plaque, or both. The plaque sample seems a particularly appropriate method because the tooth surfaces constitute the natural habitat of mutans streptococci (Gibbons and

van Houte, 1975). In the present study a toothpick was used to remove the plaque sample. It has been shown that more surfaces are found to be positive for mutans streptococci when the sample is taken with a toothpick rather than with a carver, needle or floss (Wennerholm et al., 1995b).

Counts of mutans streptococci can usually be correlated with caries experience; children with caries have higher counts than caries-free children (Hallonsten et al., 1995; Zoitopoulos et al., 1996). No child in the present study had carious teeth.

Grindefjord et al. (1991) found that amounts of salivary mutans streptococci in 1-year-old children were associated with mothers' education; children whose mothers' education had been for under 6 years were more likely to have early colonisation. We found no association between mothers' education and counts of mutans streptococci in plaque. It is of note that the majority of mothers (69%) had higher education or a professional degree; 31% had high school education only.

The amount of salivary mutans streptococci has been significantly correlated with oral hygiene (Gingival Index) in 12-year-old children (Beighton et al., 1996); similarly, we found that poor oral hygiene was significantly associated with the detection of mutans streptococci. Children whose teeth had been brushed by 12 months of age were less likely to have detectable mutans streptococci in their plaque. Almost all children who were brushing or had had their teeth brushed at 12 months were using fluoride toothpaste. Fluoride is known to decrease the rate of sugar uptake and acid production by plaque bacteria through the inhibition of enzymes involved in glycolysis and in the synthesis of intracellular storage material (Marsh and Martin, 1994). The occurrence of salivary mutans streptococci declines in association with the regular use of fluoride tablets (Raitio et al., 1995). The use of fluoride toothpaste by children whose teeth had been brushed by 12 months of age could explain the low counts of mutans streptococci in their plaque, but this possibility requires further investigation. Some investigators have not found a correlation between tooth brushing, use of fluoride and early detection of mutans streptococci (Grindefjord et al., 1991).

The infant's diet undergoes remarkable changes as they grow older; the diet at 4–6 months of age is very different from that at 9–12 months (Department of Health and Social Security, 1994) and it is therefore important to investigate the diet at each stage of growth and development. Weaning usually extends over 4–6 months of age; the teeth start to erupt at approx. 6 months of age and it is important to investigate the dietary pattern at eruption and then continuously, to detect any effects of diet on future dental health. The present dietary analysis allowed investigation of the frequency of consumption of non-milk extrinsic sugars, which may be present in foods and drinks given during infancy, at three different stages during infancy/early childhood. The mean frequency of consumption of foods/drinks containing non-milk extrinsic sugars comprised 46, 60 and 67% of the mean total eating/drinking events at 6, 12 and 18 months of age, respectively.

Because only a small number of the children had detectable mutans streptococci, our ability adequately to conduct subgroup analyses and reasonably to evaluate the presence of interaction effects between the various independent variables was limited. An association between diet and caries and between diet and the microflora has been demonstrated under abnormal and extreme conditions (Rugg-Gunn, 1993). Any association between dietary intake and the microflora in populations consuming a conventional diet and living in a normal free manner is difficult to establish, owing to the many extraneous factors that may confound statistical analysis.

Although the consumption of sugar-containing beverages has been correlated with the presence of mutans streptococci in infants (Grindefjord et al., 1991), we could not confirm this here. In agreement with other investigators (Roeters et al., 1995; Beighton et al., 1996), we found a very low correlation between frequency of consumption of sugars and counts of mutans streptococci. The observed correlation ($r = 0.2$) between diet, number of sugar intakes and number of mutans streptococci in the plaque or saliva of children aged 2–5 years (Roeters et al., 1995) was similar to our finding ($r = 0.15$) and neither was significant at $P < 0.05$. The low correlation detected here could be due to errors associated with measuring habitual dietary intakes (Rugg-Gunn et al., 1984; Hackett et al., 1985). Nevertheless, the detection of mutans streptococci was significantly correlated with frequency of eating/drinking events at 12 months of age ($P < 0.001$), which contrasts with the results of another study (Beighton et al., 1996) in which no significant correlation between salivary counts of mutans streptococci and total eating/drinking events was found in a group of 12-year-old English children; this difference requires further investigation. Squaring the correlation coefficients provides an estimate of the amount of the variance in the counts of mutans streptococci that is explained by the independent variables (Altman, 1991): the mean frequency of total eating/drinking events and the mean frequency of foods and drinks containing non-milk extrinsic sugars explained only 16 and 2% of the variances, respectively.

The multiple logistic analysis indicated the importance of starting brushing immediately after tooth eruption and of restricting the frequency of eating/drinking events in reducing mutans streptococci in the plaque of infants. We conclude that poor oral hygiene in children consuming an unrestricted diet could influence their plaque counts of mutans streptococci.

Acknowledgements

We are grateful to parents and children who made this study possible. The study was supported by The Sugar Bureau and Cow & Gates Ltd.

References

Altman, D.G., 1991. Practical Statistics for Medical Research. Chapman and Hall, London.

Beighton, D., Adamson, A., Rugg-Gunn, A., 1996. Association between dietary intake, dental caries experience and salivary bacterial levels in 12-year-old English schoolchildren. Arch. Oral Biol. 41 (3), 271–280.

Carlsson, J., Grahnén, H., Jonsson, G., Winker, S., 1970. Early establishment of *Streptococcus salivarius* in the mouths of infants. J. Dent. Res. 49, 415–418.

Carlsson, J., Grahnén, H., Jonsson, G., 1975. Lactobacilli and streptococci in the mouth of children. Caries Res. 9, 333–339.

Catalanotto, F.A., Shaklair, I., Keene, H., 1975. Prevalence and localisation of *Streptococcus mutans* in infants and children. J. Am. Dent. Assoc. 91, 606–609.

Caufield, P.W., Cutter, G.R., Dasanayake, A.P., 1993. Initial acquisition of mutans streptococci in infants: evidence for a discrete window of infectivity. J. Dent. Res. 72, 37–45.

Department of Health and Social Security, UK, 1989. Dietary Sugars and Human Diseases. Report on health and social subjects: 37. HMSO, London.

Department of Health and Social Security, UK, 1994. Weaning and Weaning Diet. Reports on health and social subjects: 45. HMSO, London.

Eronat, R., Eden, E., 1992. A comparative study of some influencing factors of rampant or nursing caries in pre-school children. J. Clin. Pediatr. Dent. 4, 275–279.

Fujiwara, T., Sasada, E., Mima, N., Ooshima, T., 1991. Caries prevalence and salivary mutans streptococci in 0–2-year-old children of Japan. Community Dent. Oral Epidemiol. 19, 151–154.

Gibbons, R., van Houte, J., 1975. Dental caries. Ann. Rev. Med. 26, 121–135.

Gold, O., Jordan, H.V., van Houte, J., 1973. A selective medium for *Streptococcus mutans*. Arch. Oral Biol. 18, 1357–1464.

Grindefjord, M.D., Dahllöf, G., Wikner, S., Hojer, B., Modéer, T., 1991. Prevalence of mutans streptococci in 1-year-old children. Oral Microb. Immunol. 6, 280–283.

Habibian, M., Roberts, G.J., Lawson, M., Stevenson, R., Harris, S., 2001. Dietary habits and dental health over the first 18 months of life. Community Dent. Oral Epidemiol. 29, 239–246.

Hackett, A.F., Appleton, D.R., Rugg-Gunn, A.J., Eastoe, J.E., 1985. Some influences on the measurement of food intake during a dietary survey of adolescents. Hum. Nutr. Appl. Nutr. 39A, 167–177.

Hallonsten, A.-L., Wendt, L.K., Mejare, I., Birkhed, D., Hakansson, C., Lindvall, A., Edwardsson, S., Koch, G., 1995. Dental caries and prolonged breast feeding in 18-month-old Swedish children. Int. J. Paediatr. Dent. 5, 149–155.

Hamada, S., Slade, H.D., 1980. Biology, immunology and cariogenicity of *Streptococcus mutans*. Microbiol. Rev. 40, 331–384.

Kalsbeek, H., Verrips, G.H., 1994. Consumption of sweet snacks and caries experience of primary school children. Community Dent. Health 28, 477–483.

Kleemola-Kujala, E., 1978. Oral hygiene and its relationship to caries prevalence in Finish rural children. Proc. Finn. Dent. Soc. 74, 76–85.

Kleinberg, I., 1970. Formation and accumulation of acid on the tooth surface. J. Dent. Res. 49, 1300–1316.

Krasse, B., Edwardsson, S., Svensson, I., Trell, I., 1967. Implantation of caries-inducing streptococci in the human oral cavity. Arch. Oral Biol. 12, 231–236.

Loesche, W.J., 1986. Role of streptococcus in human dental decay. Microbiol. Rev. 50, 353–380.

Marsh, P., Martin, M., 1994. Oral Microbiology, 3rd Edition. Chapman and Hall, London, p. 67.

Muller, M., 1996. Nursing-bottle syndrome: risk factors. ASDC J. Dent. Child. 42–50.

Raitio, M., Möttönen, M., Uhari, M., 1995. Toothbrushing and the occurrence of salivary mutans streptococci in children at day care centres. Caries Res. 29, 280–284.

Registrar General, 1993. Office of Population Censuses and Surveys. Classification of occupation. Report for Great Britain (Part 1). HMSO, London.

Ripa, L.W., 1978. Nursing habits and dental decay in infants: 'nursing bottle caries'. ASDC J. Dent. Child. 45, 274–278.

Ripa, L.W., 1988. Nursing caries: a comprehensive review. Pediatr. Dent. 10, 268–282.

Roeters, F., van der Hoeven, J.S., Burgersdijk, RC.W., Schaeken, M.J.M., 1995. Lactobacilli, mutans streptococci and dental caries: a longitudinal study in 2-year-old children up to the age of 5 years. Caries Res. 29, 272–279.

Rugg-Gunn, A., 1993. Nutrition, Diet and Dental Health. Oxford Medical Publications, London.

Rugg-Gunn, A.J., Hackett, A.F., Appleton, D.R., Jenkins, G.N., Eastoe, J.E., 1984. Relationships between dietary habits and caries increment assessed over 2 years in 405 English adolescent schoolchildren. Arch. Oral Biol. 29, 983–992.

Sclavos, S., Porter, S., Seow, W., 1988. Future caries development in children with nursing bottle caries. J. Pedodont. 13, 1–10.

Tinanoff, N., O'Sullivan, D.M., 1997. Early childhood caries: overview and recent findings. Pediatr. Dent. 19 (1), 12–19.

van Houte, J., 1994. Role of microorganisms in caries aetiology. J. Dent. Res. 7 (2), 87–96.

van Houte, J., Gibons, G., Butera, C., 1982. Oral flora of children with 'nursing bottle caries'. J. Dent. Res. 61, 382–385.

Wendt, L.K., Hallonsten, A.-L., Koch, G., 1991. Dental caries in 1- and 2-year-old children living in Sweden. Part I. A longitudinal study. Swed. Dent. J. 15, 151–156.

Wennerholm, K., Birkhed, D., Emilson, C.G., 1995a. Effects of sugar restriction on *Streptococcus mutans* and *Streptococcus sobrinus* in saliva and dental plaque. Caries Res. 29, 54–61.

Wennerholm, K., Lindquist, B., Emilson, C.-G., 1995b. The toothpick method in relation to other plaque sampling technique for evaluating mutans streptococci. Eur. J. Oral Sci. 103, 36–41.

World Health Organisation, 1987. Oral Health Surveys: Basic Methods, 3rd Edition. WHO, Geneva.

Zoitopoulos, L., Brailsford, S., Gelbier, S., Ludford, R., Marchant, S., Beighton, D., 1996. Dental caries and caries-associated microorganisms in the saliva and plaque of 3- and 4-year-old Afro-Caribbean and Caucasian children in south London. Arch. Oral Biol. 41 (11), 1011–1018.