Australian Dental Journal
The official journal of the Australian Dental Association

Australian Dental Journal 2014; 59: 106–113

doi: 10.1111/adj.12145

Caries experience, mutans streptococci and total protein concentrations in children with protein-energy undernutrition

T Rodrigues Ribeiro,* K Shangela da Silva Alves,* AC de Miranda Mota,* D Pereira Costa,* C Barreto Mano de Carvalho,* CF Santos,† AJ Monteiro,* CSR Fonteles*

*Federal University of Ceará, Fortaleza, Brazil.
†State University of Ceará, Fortaleza, Brazil.

ABSTRACT

Background: The highest prevalence of protein-energy undernutrition is observed during early childhood, being also a time in which the presence of dental caries can be unusually aggressive. The present study aimed to verify if different levels of undernutrition could influence the risk of early childhood caries (ECC), in the presence of other predisposing factors.

Methods: One hundred and twenty undernourished 12–70 month old children, with or without ECC, were selected. Undernourished children were classified as being mildly, moderately or severely undernourished. All children were examined for determination of decayed, missing and filled surfaces (dmfs). Total protein concentration in saliva was analysed by the Bradford method. For microbiological analysis, mitis salivarius-bacitracin agar medium was used. A binary logistic regression model was applied to test the simultaneous influence of different variables over caries experience.

Results: The risk of ECC was significantly higher with an increase in age ($p = 0.000$) and mutans streptococci counts ($p = 0.032$). Comparisons with the normal-weight group showed that mildly ($p = 0.004$) and severely undernourished children ($p = 0.037$) had a higher risk of experiencing ECC, but this risk was not significantly elevated among moderately undernourished children ($p = 0.158$).

Conclusions: Our results suggest that mildly and severely undernourished children have an increased risk of experiencing dental caries. Age is highly associated with the disease in this population.

Keywords: Child, dental caries, malnutrition, salivary proteins and peptides, *Streptococcus mutans*.

Abbreviations and acronyms: AAPD = American Academy of Pediatric Dentistry; dmfs = decayed, missing and filled surfaces; ECC = early childhood caries; IPREDE = Institute for Preventing Undernutrition and Exceptionality; PEU = protein-energy undernutrition; TPC = total protein concentration.

(Accepted for publication 15 April 2013.)

INTRODUCTION

Globally, early childhood caries (ECC) and protein-energy undernutrition (PEU) remain significant public health issues in many different countries. PEU is a condition that results from inadequate consumption of nutrients, interference in oral intake and absorption, or from the body's inability to utilize the ingested nutrients, due to disease or increased nutritional needs.[1] The time encompassing gestation through to age 5 is the most vulnerable nutritional phase in the human life cycle. Therefore, rapid growth and the development of the immune system against infections determine unique dietary needs, generating a higher impact at this stage than in latter developmental periods.[1] Unfortunately, the highest prevalence of PEU is observed during early childhood, especially between the ages 2 and 5,[2] a time in which the presence of dental caries can be unusually aggressive, with the potential for increasing feeding difficulties, added risk of infection,[3] impacting on weight gain[4] and the child's well-being.[5]

According to the American Academy of Pediatric Dentistry (AAPD),[6] a child should be diagnosed as having ECC if at least one carious cavitated or non-cavitated dental surface is present, missing (due to caries) or filled (dmfs), on a primary tooth, right after dental eruption until 71 months of age. In addition, any sign of smooth-surface caries before age 3 indicates the presence of severe ECC. Caries pattern can be affected by age, dental morphology, eruption sequence, diet and behavioural factors.[7,8] Nutritional deficiency has an important influence over dental development and disease. It has previously been demonstrated that children with PEU have delayed dental eruption and exfoliation, higher prevalence of enamel hypoplasia, salivary gland hypofunction, and higher caries experience in the primary[9] and permanent[10] dentitions. The present study has aimed to verify if different levels of undernutrition can be considered indicators of caries risk during early childhood, in the presence of other factors, such as age, gender, salivary MS levels and total protein concentration in saliva, to test the hypothesis that undernourished children would be more susceptible to dental caries, expressing higher caries experience.

MATERIALS AND METHODS

Population and study design

This study was conducted over a one-year period, where patient recruitment was carried out between April 2006 and February 2007. A total of 800 12–70 month old children from both genders, with a medical history consistent with the presence of undernutrition, and the absence of other systemic derangements, diseases, hereditary or congenital malformations were screened to participate in this study, at the Institute for Preventing Undernutrition and Exceptionality (IPREDE), the reference centre in the treatment of undernutrition in Ceará, Brazil. One hundred and twenty children with PEU were selected and enrolled as study participants. These children were divided into three different groups of undernourishment levels, according to WHO 2006 growth standards:[11] mild (< -1 to > -2 Z-score), moderate (< -2 to > -3 Z-score) or severe (< -3 Z-score) PEU, for instance undernutrition grades I (GI, n = 31); II (GII, n = 59), or III (GIII, n = 30), respectively. The Z-score (number of standard deviations a child is from the mean) for anthropometric measurements was calculated using Epi Info version 6.0 (Centers for Disease Control, Atlanta, USA) by inserting child's date of birth, gender, weight and height/length. Forty-eight normal-weight children who attended the Pediatric Dental Clinic at the Federal University of Ceará, from both genders, at high caries risk (low socio-economic status, parents with low-educational level, and absence of previous dental visits), within a similar age range (12–82 months age), caries status (caries free, n = 22; ECC, n = 25) and socio-economic background were selected to participate as controls (GN). These children resided in the same towns and communities as the undernourished population. In order to control for possible confounders in all studied groups (normal-weight and PEU children) and insure a high caries risk population, children that consumed less than three sucrose-containing meals/day (decreasing caries risk), breastfed past 6 months, or had overnight bottle feeding habits (increasing caries risk, but acting as potential confounders) were excluded from the study.

This study was conducted according to the guidelines outlined in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Ethics Committee of the Federal University of Ceará Medical School, Brazil. Written informed consent was obtained from all parents or legal guardians, prior to patient enrolment in the study.

Saliva samples

Unstimulated whole saliva was collected from all participants, for 60 seconds, by using a disposable cannula, placed into plastic tubes, centrifuged at 3000 x g for 5 minutes, at 32 ºC.[12] A corresponding volume of 100–200 μL of the supernatants was extracted, lyophilized and stored at –20 ºC for posterior total protein analysis. Subsequently, children were asked to chew on a standard piece of Parafilm® for 60 seconds in order to stimulate salivary secretion and release plaque into the salivary fluid. Saliva was then collected with a disposable plastic cannula and stored in sterile Ependorfs®. Samples were transported to the laboratory for microbiological analysis in a hermetically sealed case containing ice, and analysed no longer than 2 hours after collection.[13] In order to control for circadian rhythm, saliva samples were collected between 8 am and 11.30 am.

Examination

After brushing with a pea-sized amount of fluoridated toothpaste, teeth were dried and a dental examination was undertaken using a mirror and probe under a dental unit light. Prior to study initiation, a calibration process was carried out in order to minimize diagnostic errors regarding caries experience between groups. Only one examiner evaluated all patients, being 20 children examined prior to study initiation. These children were re-examined after a 7-day interval to verify diagnostic criteria and to access intra-examiner reproducibility. Kappa statistics was calculated according to the following formula: K = Po-Pe/ 1-Pe, where: Po = ratio of observed agreements; Pe = ratio of expected agreements. A mean Kappa value of 0.81 was obtained. Caries experience was determined through the visual/tactile method. A dental explorer was gently used across dental surfaces to clean the area before examination and verify the degree of surface roughness. Non-cavitated lesions (initial caries lesions) were identified when the toothbrushed and air-dried surfaces presented a 'chalky-white' appearance. Thus, with a loss of translucency of the enamel, a greater surface roughness was usually observed. These white spot lesions were commonly detected in the cervical and buccal surfaces of teeth.[14–16] Hypoplastic/hypomineralized lesions were defined as a break in the continuity of the enamel, in the form of pits, grooves, or missing enamel. Enamel opacity was defined as a change in the translucency of enamel, without a break in the enamel continuity.

Caries experience was determined by calculating the number of decayed, missing and filled surfaces (dmfs scores). Participants were separated into two different groups: children who had (dmfs > 0) and children who had not experienced dental caries (dmfs = 0). In order to control for confounders, the number and location of erupted and hypoplastic teeth were recorded for each child. Tooth surface was the unit used to register carious lesions. A tooth was considered erupted if any part of the crown had penetrated the gingival tissues. Children having at least one affected primary tooth surface were considered to have ECC. Cavitated and non-cavitated (white spot) lesions were recorded, and severity of ECC was registered based on AAPD guidelines (2010–2011).[6] Hence, severe ECC was considered to be present in any of the following situations: (1) before age 3, when any sign of smooth-surface caries was identified; (2) from ages 3–5, when there was a primary upper anterior smooth-surface dmf ≥1; or (3) when dmfs scores ≥4 (age 3), ≥5 (age 4) or ≥6 (age 5) were noted.

Microbiological analysis

Stimulated whole saliva samples were serially diluted in saline, establishing dilutions of 1:100 and 1:1000. Aliquots of 100 μL from the different dilutions were placed in duplicates on mitis salivarius bacitracin agar plates (Difco, Detroit, Michigan, USA).[17] The plates were incubated at 37 °C for 48 hours in jars under microaerofilic conditions. Representative colonies with morphological characteristics of MS were counted, isolated and biochemically confirmed to be MS utilizing the mannitol fermentation test. Bacterial counts were expressed as colony forming units (CFU) per millilitre of saliva. According to the different levels of contamination, children were considered as having low (undetectable CFU), moderate (1–99 000 CFU) or high (>100 000 CFU) salivary MS counts.[18]

Total protein analysis

Unstimulated whole saliva was analysed for total protein concentration (TPC) by the Coomassie blue method,[19] with serum bovine albumin (Sigma Chemical Co., St Louis, MO, USA) used as a standard. The lyophilized saliva samples were reconstituted by adding 50 μL of Milli-Q water, and homogenized using a mechanical agitator (Vortex®, AP-56, Phoenix, São Paulo, Brazil). Subsequently, 10 μL of the reconstituted sample was withdrawn, and 2.5 mL of Bradford solution was added, re-homogenized and put to rest for 10 minutes so as to take an absorbance reading with a spectrophotometer (Ultraspec 1100, Pharmacia, England). An absorbance of 595 nm was used.

Statistical analysis

Data were analysed using SPSS for Windows (Version 17.0, 2008; SPSS Inc., Chicago, IL, USA). Kruskal–Wallis test was applied to verify any existing differences in age, MS counts, TPC and dmfs scores between GI, GII, GIII and GN; as well as for comparisons of these variables among caries-free children and those with and without severe ECC. Mann–Whitney test was used for comparisons of age, MS counts, TPC and dmfs scores between genders and between GI and GII, GN and GI/GII, GIII and GI/GII, GN and GIII. For comparisons between genders among caries-free children and those with and without severe ECC chi-square test was used. To verify monotonic relation between the different variables Spearman rank correlation coefficient was applied. To test the simultaneous influence of different variables over caries experience, a binary logistic regression model was used. Comparisons of ages of children with (dmfs >0) and without (dmfs = 0) caries in GI, GII, GIII and GN, and comparisons of ages between groups among children with caries were done by applying Student's *t*-test. In order to minimize the great asymmetries observed with MS counts, a logarithm of this variable was used in the binary logistic regression model, and for comparisons using Student's *t*-test. Only differences with a p-value <0.05 were considered as statistically significant.

RESULTS

Study demographics

Eight hundred undernourished children and 400 normal-weight children were screened to participate in the study, of which 164 undernourished children and 48 normal-weight children met the inclusion criteria. However, 42 undernourished children were judged by parents as being too young, and unable to cooperate, thus parents chose not to sign the informed consent. Two undernourished children (GI and GII), and one normal-weight child were removed from the study due to a lack of cooperation during saliva collection. The final study sample consisted of 120 undernourished children from both genders (equal distribution), with (n = 44) or without (n = 76) ECC, and 47 normal-weight children within the same age range and caries status (caries free, n = 22; ECC, n = 25).

GIII children were the youngest among all groups (GI/GII, p = 0.006; GN, p = 0.000). When comparing ages (12–70 month aged children) between genders in the different undernutrition levels, boys GIII were found to be significantly younger than girls (p = 0.003). However, TPC, MS levels and dmfs scores did not differ between genders in GN, GI, GII or GIII. Furthermore, no significant association was observed between genders and severity of ECC (p = 0.668). Comparison between ages among children who were caries-free, and children with and without severe ECC demonstrated a statistical significant difference (p = 0.000), for instance severe ECC was more often present among children at older ages. This finding did not depend on the child's nutritional status. A statistically significant positive correlation was found between age and MS counts (p = 0.031); age and dmfs scores (p = 0.000); age and TPC (p = 0.008).

Caries experience and undernutrition

Forty-five undernourished (37.5%) and 25 (53.2%) normal-weight children presented a history of dental caries (dmfs > 0). A total of seven undernourished children (15.5%) presented carious surfaces on the anterior teeth only, 22 (49%) children presented carious surfaces on the posterior teeth only, and 16 (35.7%) participants had carious surfaces on both the anterior and posterior dentitions. In GN, carious surfaces affected only the anterior teeth in a total of three (12%) children, only the posterior teeth in 15 (60%), while seven (28%) children had carious surfaces affecting both the anterior and posterior dentitions (Table 1).

The second primary molar was the most affected primary tooth by dental caries among undernourished and GN children, followed by the first primary molar and upper central incisor. Lower incisors were not affected by dental caries in any of the studied groups. White spot lesions were identified in only seven (15.9%) undernourished and six (24%) GN children. Only three (2.5%) undernourished children were identified as having enamel hypoplasia, one in GII (primary upper central incisors were affected) and two in GIII (upper canines and molars). In contrast, six (12.5%) GN children presented with 11 affected teeth, consisting of primary molars, central incisors and canines.

Caries experience and mutans streptococci levels

MS was present in the saliva of 69 (57.5%) children in the undernourished groups (Table 2), of which 30 (25%) children were moderately and 39 (32.5%) were highly contaminated with MS. In GN, 31 (65.9%) children had detectable levels of MS. In this group, 12

Table 1. Number of carious teeth distributed by age group and nutritional status

Age[§] (Months)	Upper																			
	CI[a]				LI[b]				C[c]				1st M[d]				2nd M[e]			
	N[f]	I[g]	II[h]	III[i]	N	I	II	III	N	I	II	III	N	I	II	III	N	I	II	III
12-23*	-	-	-	2	-	-	-	2	-	-	-	1	-	-	-	-	-	-	-	-
24-35**	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36-47***	-	4	4	1	-	-	2	2	-	-	1	-	2	-	2	2	3	2	4	-
48-59****	1	4	9	2	-	1	-	3	1	-	-	2	2	3	2	7	5	3	7	5
60-70*****	4	-	2	-	1	1	-	-	2	1	3	-	4	1	3	-	5	4	11	-
	Lower																			
	CI				LI				C				1st M				2nd M			
	N	I	II	III	N	I	II	III	N	I	II	III	N	I	II	III	N	I	II	III
12-23*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24-35**	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-
36-47***	-	-	-	-	-	-	-	-	-	-	-	-	3	2	2	3	6	5	10	-
48-59****	-	-	-	-	-		-	-	2	-	-	2	2	4	4	7	4	4	8	8
60-71*****	-	-	-	-	-	-	-	-	-	-	-	-	3	3	4	-	7	5	10	-

[§]The maximum number of erupted teeth specified for each age range at the time of examination. Eruption of the primary dentition did not end prior to 36 months age for undernourished children (N, I, II, III). Normal-weight children finalized eruption prior to 36 months age.*Presence of central incisors, lateral incisors and canines; **Presence of central incisors, lateral incisors, canines and mandibular first molars; ***;****; *****Presence of full primary dentition; [a]CI=central incisors, [b]LI=lateral incisors, [c]C=canines, [d]1stM=first primary molar, [e]2ndM=second primary molar; [f]N=normal-weight children, [g]I=mildly undernourished children, [h]II=moderately undernourished children, [i]III=severely undernourished children.

Table 2. Age, MS counts, total protein concentration (TPC), dmfs scores and number of teeth of caries-free (CF) and Early Childhood Caries (ECC) children distributed by nutritional status. Data expressed in mean (M) ± standard deviation (SD), median (Minimum-Maximum)

Groups	Values	Age		Teeth[a]		MS Counts		dmfs		TPC	
		CF	ECC	CF	ECC	CF	ECC	CF	ECC	CF	ECC
GN	*M ± S.D*	56.7 ± 17.8	61.4 ± 11.2	19.5 ± 2.3	20 ± 2	48 947 ± 117 673	122 160 ± 205 453	0	4.0 ± 5.6	1.6 ± 0.6	1.9 ± 0.7
	Md (min – max)	57.5 (12-58)	60 (44-82)	20 (15-24)	20 (15-24)	14 250 (0-550 000)	4075 (0-550 000)	0	2 (1-21)	1.7 (1.1-3.5)	1.5 (0.7-3.2)
N		22	25	22	25	22	25	22	25	22	25
GI	*M ± S.D*	29.9 ± 11. 9	43.1 ± 13.4	17.37 ± 3.70	16.85 ± 5.31	280 617 ± 477 976	134 750 ± 22 182	0	4.8 ± 5.8	1.5 ± 1.2	1.3 ± 0.5
	Md (min – max)	28.5 (13-53)	43 (26-64)	20 (11-20)	18.5 (2-20)	90 250 (0-1 640 000)	30 500 (0-740 000)	0	2 (1-19)	1.2 (0.5-5.3)	1.1 (0.7-2.3)
N		17	15	17	15	17	15	17	15	17	15
GII	*M ± S.D*	30 ± 14.3	54 ± 9.0	14.9 ± 5.7	20 ± 0	796 643 ± 1 586 634	481 855 ± 1 532 599	0	5 ± 4.9	1.1 ± 0.7	1.2 ± 0.5
	Md (min – max)	27 (12-64)	55 (37-70)	16 (2-20)	20 (20-20)	91 000 (0-6 140 000)	0 (0-8 240 000)	0	4 (1-17)	0.9 (0-3.0)	1.0 (0.6-2.1)
N		38	20	38	30	38	20	38	20	38	20
GIII	*M ± S.D*	23.6 ± 12.6	41.4 ± 16.4	11.4 ± 6.2	18.1 ± 2.8	111 976 ± 386 849	381 666 ± 569 709	0	12.0 ± 22.6	1.7 ± 1.0	1.9 ± 0.7
	Md (min – max)	18 (12-51)	43 (15-58)	11.5(2-20)	19.5 (12-20)	0 (0-1 785 000)	144 000 (0-1 620 000)	0	6 (1-72)	1.3 (0.7-4.7)	1.8 (0.9-3.0)
N		21	9	21	9	21	9	21	9	21	9

(38.7%) children presented high MS concentrations, whereas 19 (61.3%) children had moderate salivary MS levels. Comparisons between children without ECC (dmfs = 0), and children with caries showed a significant difference in MS counts (p = 0.001) and caries experience (p = 0.000). MS counts and dmfs scores were positively correlated (p = 0.000). Hence, an increase in MS counts was observed when caries became more severe, regardless of the child's nutritional status. Comparison of children with different levels of undernutrition (GI, GII, GIII) and the GN group showed no statistically significant differences in MS counts (p = 0.541) or caries experience (p = 0.609).

Total protein concentration

Comparisons between children without ECC (dmfs = 0), and children with caries showed no significant difference in TPC between groups (p = 0.602). TPC did not correlate with caries experience (p = 0.565) or MS levels (p = 0.216) among healthy or undernourished children. TPC (p = 0.204) and caries experience (p = 0.395) did not differ between GI and GII. Nevertheless, GIII children presented higher TPC than children in GI/GII groups (p = 0.000), but no statistical difference in TPC was observed when comparing GIII and GN groups (p = 0.531).

Multivariate analysis of caries experience

When using a binary logistic regression model for simultaneous analysis of all variables, gender (p = 0.709) and TPC (p = 0.272) did not significantly contribute with caries experience, but age (p = 0.000), MS count (p = 0.032) and nutritional status (p = 0.032) significantly contributed with caries experience (Table 3). The risk of experiencing dental caries was significantly higher with an increase in age, as

Table 3. Analysis of different variables and their association with caries experience using a Binary Logistic Regression Model (P < 0 05)

Variables	Statistics			
	B	Wald	DF	P-value
Gender	−0.142	0.139	1	0.709
Undernutrition	–	8.831	3	0.031
GI[a]	1.857	8.190	1	0.004
GII[a]	0.755	1.995	1	0.158
GIII[a]	1.466	4.343	1	0.037
Age	0.081	29.099	1	0.000
MS Log Counts	0.071	4.593	1	0.032
TPC	−0.265	1.207	1	0.272

B, Regression coefficient
DF, Degree of freedom
[a]Comparisons made with GN

well as with an increase in MS counts. Caries risk was also higher in GI ($p = 0.004$) and GIII ($p = 0.037$), but was not significantly elevated in GII ($p = 0.158$), when comparisons were made with GN.

DISCUSSION

In the present study, age played a major role in dental caries experience. Thus, the older the child, the higher the risk of caries development. An association between the prevalence and severity of ECC, and an increase in age has been recently described among children with different nutritional status.[20] Among undernourished children, this association has been formerly linked to a delayed dental eruption. Alvarez *et al.*[21] while studying the association between chronic undernutrition and dental caries, described a shift to the right on the deft-versus-age curve. Further studies confirmed these findings.[9] However, plotting dmfs scores as a function of age in the present study did not demonstrate the previously described shift to the right of the age distribution curve of caries among undernourished children. We believe the presently observed influence of age over caries experience could be partly explained by delayed eruption. Nevertheless, it is also a factor of a greater picture, where undernourishment could lead to a deficit in the child's ability to immunologically defend against ECC. If this is a fact, the longer the child remains in an undernourished state, which happens with chronically undernourished children at older ages, the higher the risk of dental caries experience.

A limitation of this study was its sample size. The possible influence of potential confounders determined the establishment of strict inclusion/exclusion criteria, which may explain the limited number of participating children. For instance, children who breastfed past age 6 months, as well as those without very high consumption of sucrose-containing liquids/foods (≤3 sucrose-containing meals/day) were excluded from the study. Undernourished children consumed a great variety of junk foods, but insufficient foods/drinks with real nutritional value. Nutritionists' recommendations focused on the need for dietary modification, and instructions to intensify consumption of milk or formula, rice, beans and oil (to increase caloric intake), allowing adequate growth and development. These children received free milk/formula every other week, in addition to rice, beans and oil to supplement their diets. This protocol was followed for all children, regardless of their level of undernutrition. Hence, this study population was constituted by children at very high risk for experiencing dental caries, and this risk factor was mainly attributed to: (1) diet; (2) socio-economic status; (3) parents' level of education; and (4) nutritional status.

In the present study, the risk of experiencing dental caries was elevated among mildly and severely undernourished children, but the moderately undernourished group was not at a significantly higher risk of developing the disease. We believe this phenomenon may be explained by two different factors: (1) GN children constituted by definition a high caries risk group; (2) GII children with caries were at a similar age group, when compared to GN, unlike GI and GIII-children who were at a younger age range. These similarities may have masked the high-risk category of GII in relation to GN, in the presence of other risk factors, in a multivariate statistical model. It must also be noted that the level of significance ($p = 0.158$) observed for GII does not exclude the possibility of observing a higher level of caries risk in a larger patient population. Oliveira *et al.*[22] have investigated the association of nutritional status and dental caries in Brazilian preschool children. Their results showed an increased risk of developing dental caries in children with low Z-scores in body mass index-for-age and weight-for-height. The authors used the WHO Child Growth Standards Reference to evaluate nutritional status, but did not classify different levels of undernutrition. Therefore, differences in caries risk associated with mild, moderate and severe undernutrition could not be observed. An association between nutritional status and dental caries among elementary school children has been previously described;[23] this phenomenon was also observed in the permanent dentition.[24] Since a reduction in salivary flow rate,[25] decreased buffering capacity and changes in salivary protein activity of undernourished children have been previously described,[22] we believe future investigation of the biochemical aspects of saliva in undernourished populations may give a better understanding of the different systemic aspects involved in the caries risk mechanism of undernourished children during early childhood.

Previously, Li *et al.*[26] assessed salivary MS levels and nutritional status among 3–4 year old children. The author's main objective was to investigate the association between salivary MS levels and enamel hypoplasia. However, dental caries was also evaluated and a significant association between MS levels and caries was detected, but no significant correlation was found between numbers of MS and body height or body weight. Johansson *et al.*[20] studied the effect of chronic PEU on salivary flow and caries susceptibility in a group of 68, 8–12 year old children, of which 34 were classified as being severely/moderately undernourished, and the remaining children were diagnosed as part of the nourished/mildly malnourished group. When considering the number of highly contaminated ($>10^6$ UFC/mL) children in both groups, 74% of the severely/moderately and 65% of the nourished/mildly undernourished children had high levels of MS in saliva. In their study all participants were contaminated with MS, and no difference in salivary MS levels was found between groups. In the present study, mean levels of salivary MS were not found to be as elevated as the ones observed by Johansson *et al.*,[20] since not all participants were contaminated with MS, and high concentrations of MS were only observed in approximately 30% of the undernourished population. However, in agreement with our findings, nutritional status alone did not influence MS contamination in any of these previous studies.

Since we have formerly observed an association between TPC and undernutrition (data not shown), we have measured TPC in saliva in an attempt to verify if higher or lower TPC in children with different grades of undernutrition was capable of influencing the risk of experiencing dental caries. A variety of factors are capable of influencing TPC measurements, such as age,[27] geographic location, nutritional habits and the method of saliva sampling.[28] Apparently, TPC is not influenced by dental eruption[27] and no difference between genders has been previously described. Our results demonstrated a lack of correlation between TPC and ECC, as has been previously reported by Farias and Bezerra[29] among healthy children. The authors found no differences in TPC among 12–47 month old children with or without dental caries. Conversely, Kargül *et al.*[30] observed a linear rise in TPC with an increasing number of carious surfaces. We believe this difference was possibly due to the wide discrepancy in children's age, since over 50% of their sample included 6–13 year old children and age factor may significantly influence TPC.[28]

Dental caries is a multifactorial disease, therefore simultaneous analysis of all possible risk factors was needed in order to establish the ability of each individual variable to significantly contribute with caries experience. This type of analysis revealed a significant contribution given by MS counts and the child's nutritional condition in the development of caries. Furthermore, the presence of PEU was a relevant predisposing factor in the development and manifestation of the disease. Cleaton-Jones *et al.*[31] studied the relationship between nutritional status and dental caries in 2728 children aged 4–5 years. Although a statistically significant trend in the increase of dmfs scores with increased wasting (low weight-for-height) was observed, no significant rise in caries prevalence was noted in this population. Despite demonstrating a significant association between wasting (low weight-for-height) and dmfs scores, the authors concluded that nutritional status was not a clinically significant factor in caries prevalence and experience. Nevertheless, in agreement with our data, previous work has suggested a relationship between PEU and increased caries experience,[9] and it has been proposed that a single mild to moderate undernutrition episode in the first year of life may result in higher caries rates in the primary and permanent dentitions.[9,10] Previously, the lack of agreement on the methods used to properly diagnose dental caries and undernutrition has been the main difficulty encountered in the comparison of our data and the data reported by others. Current understanding of dental caries as a sucrose-dependent disease, its associated risk factors, and the evidence-based malnutrition standards established in 2006 by the World Health Organization[11] may allow future studies to overcome these past limitations. In addition, very little is known on the salivary changes that intercept caries and undernutrition. Future studies should focus on identifying specific salivary characteristics that may be of interest in the development of preventive, diagnostic and/or therapeutic strategies against these diseases.

CONCLUSIONS

Our results suggest that nutritional status predisposes mildly and severely undernourished children to a higher risk of experiencing dental caries in early childhood when other risk factors are considered, such as degree of undernutrition, age and MS levels in saliva.

ACKNOWLEDGEMENTS

We are grateful to the Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP) for supporting this work.

REFERENCES

1. Dutra-De-Oliveira JE, Marchini JS. Nutritional sciences. São Paulo: Sarvier, 2003.
2. World Health Organization. Department of Nutrition for Health and Development 2010. Nutrition for health and development: a global agenda for combating malnutrition. URL: 'http://whqlibdoc.who.int/hq/2000/WHO_NHD_00.6.pdf'. Accessed June 2011.
3. Schwartz S. A one year statistical analysis of dental emergencies in a pediatric hospital. J Can Dent Assoc 1994;60:959–968.
4. Ayhan H, Suskan E, Yildirim S. The effect of nursing or rampant caries on height, body weight, and head circumference. J Clin Pediatr Dent 1996;20:209–212.
5. Low W, Tan S, Schwartz S. The effect of severe caries on the quality of life in young children. Pediatr Dent 1999;21:325–326.
6. American Academy of Pediatric Dentistry. AAPD 2010–11 definitions, oral health policies, and clinical guidelines 2010. Policy on early childhood caries (ECC): classifications, consequences, and preventive strategies. Available at: http://www.aapd.org/media/Policies_Guidelines/P_ECCClassifications.pdf.
7. Ölmez S, Uzamis M, Erdem G. Association between early childhood caries and clinical, microbiological, oral hygiene and dietary variables in rural Turkish children. Turk J Pediatr 2003; 45:231–236.

8. Shishniashvili TE, Margvelashvili VV, Suladze NN, Kobakhidze KA. Correlation between the ecological risk factors and significant index of caries in young children. Georgian Med News 2012;206:30–33.
9. Alvarez JO, Eguren JC, Caceda J, Navia JM. The effect of nutritional status on the age distribution of dental caries in the primary teeth. J Dent Res 1990;69:1564–1566.
10. Alvarez JO. Nutrition, tooth development, and dental caries. Am J Clin Nutr 1995;61:410S–416S.
11. WHO Multicentre Growth Reference Study Group. WHO child growth standards based on length/height; weight and age. Acta Paediatr 2006;Suppl 450:76–85.
12. Fonteles CS, Guerra MH, Ribeiro TR, *et al*. Association of free amino acids with caries experience and mutans streptococci levels in whole saliva of children with early childhood caries. Arch Oral Biol 2009;54:80–85.
13. Ruoff KL, Whiley RA, Beighton D. Streptococcus. In: Murray PR, Baron EJ, Jorgensen JW, Pfaller MA, Yolken RH, eds. Manual of clinical microbiology. Washington DC: ASM Press, 2003:405–421.
14. Nikiforuk G. The caries process, morphological and clinical events. In: Understanding dental caries. Etiology and mechanisms, basic and clinical aspects. New York: Ed Karger, 1985:261.
15. Giro C. Enamel hypoplasia in human teeth: an examination of its causes. JADA 1947;34:310–317.
16. Sabel N. Enamel of primary teeth–morphological and chemical aspects. Swed Dent J Suppl 2012;222:1–77.
17. Gold OG, Jordan HV, van Houte J. A selective medium for *Streptococcus mutans*. Arch Oral Biol 1973;18:1357–1364.
18. Jordan HV, Laraway R, Snirchi R, Marmel M. A simplified diagnostic system for cultural detection and enumeration of *Streptococcus mutans*. J Dent Res 1987;66:57–61.
19. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–254.
20. Johansson I, Saelström A-K, Rajan BP, Parameswaran A. Salivary flow and dental caries in Indian children suffering from chronic malnutrition. Caries Res 1992;26:38–43.
21. Alvarez JO, Lewis CA, Saman C, *et al*. Chronic malnutrition, dental caries, and tooth exfoliation in Peruvian children aged 3–9 years. Am J Clin Nutr 1988;48:368–372.
22. Oliveira LB, Sheiham A, Bönecker M. Exploring the association of dental caries with social factors and nutritional status in Brazilian preschool children. Eur J Oral Sci 2008;116:37–43.
23. Lueangpiansamut J, Chatrchaiwiwatana S, Mukatabhant B, Inthalohit W. Relationship between dental caries status, nutritional status, snack foods, and sugar-sweetened beverages consumption among primary schoolchildren grade 4–6 in Nongbua Khamsaen school, Na Klang district, Nongbua Lampoo Province Thailand. J Med Assoc Thai 2012;95:1090–1097.
24. Delgado-Angulo EK, Hobdell MH, Bernabé E. Childhood stuting and caries increment in permanent teeth: a three and a half year longitudinal study in Peru. Int J Paediatr Dent 2013;23:101–109.
25. Psoter WJ, Spielman AL, Gebrian B, St Jean R, Katz RV. Effect of childhood malnutrition on salivary flow and pH. Arch Oral Biol 2008;53:231–237.
26. Li Y, Navia JM, Caulfield PW. Colonization by mutans streptococci in the mouths of 3- and 4-year-old Chinese children with or without enamel hypoplasia. Arch Oral Biol 1994;39:1057–1062.
27. Dezan CC, Nicolau J, Souza DN, Walter LR. Flow rate, amylase activity, and protein and sialic acid concentrations of saliva from children aged 18, 30 and 42 months attending a baby clinic. Arch Oral Biol 2002;47:423–427.
28. Ruhl S, Rayment SA, Scmalz G, Hiller KA, Troxler RF. Proteins in whole saliva during the first year of infancy. J Dent Res 2005;84:29–34.
29. Farias DG, Bezerra ACB. Salivary antibodies, amylase and protein from children with early childhood caries. Clin Oral Investig 2003;7:154–157.
30. Kargül B, Yarat A, Tanboga I, Emekli N. Salivary protein and some inorganic element levels in healthy children and their relationship to caries. J Marmara Univ Dent Fac 1994;2:434–440.
31. Cleaton-Jones P, Richardson BD, Granath L, *et al*. Nutritional status and dental caries in a large sample of 4- and 5-year-old South African children. S Afr Med J 2000;90:631–635.

Address for correspondence:
Dr Cristiane Sá Roriz Fonteles
Unidade de Pesquisas Clínicas
Universidade Federal do Ceará
Laboratório de Farmacologia Metabólica
e Fisiologia Celular
Avenida José Bastos 3390, sala 106
Caixa Postal 3229
CEP 60.436-160 Fortaleza-Ce
Brazil
Email: cfontele@ufc.br