Title: Evaluation of masticatory behavior and taste sensitivity after pacifier removal in preschool children: a 1-year follow up

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Abstract

Objective The aim was to investigate the influence of pacifier removal on the development of masticatory function and taste sensitivity in preschool children.

Methods Sixty children (mean age 48.2 months) were divided into two groups: pacifier group (n=28) and a control group (n=32), which were evaluated and followed-up for a period of 12 months (at baseline, 6 months and 1 year). Masticatory and swallowing functions were assessed using the Mastication Observation and Evaluation protocol (MOE) and Orofacial Myofunctional Rating (MBGR), respectively. Detection thresholds for sucrose and urea were measured by staircase method. The two-way ANOVA mixed model was used for time*group interaction analysis.

Results MOE scores improved significantly over time in both groups, although a significant difference between groups persisted after 1 year. On the other hand, swallowing scores were significantly different at baseline, but within 1 year the scores were no longer different between groups. Chewing time and the number of cycles were not different between groups and both decreased after 1-year. Sucrose sensitivity was significantly greater in the control group at baseline and changed over time ($p<0.05$), being no longer different between groups after 6 months. Bitter sensitivity did not differ over time nor between groups.

Conclusions Detection threshold for sucrose differed significantly between children with and without pacifier habit at a mean age of 42 months. Total masticatory function did not self-correct after sucking habit removal within a one-year period.

Clinical relevance Children with pacifier habit showed important changes in masticatory function that did not self-correct 1 year after cessation of the habit, highlighting the need for prevention and habit interruption as early as possible.

Keywords: Taste Threshold. Preschool Children. Mastication. Pacifier.

Introduction

Sucking is an innate reflex driven from the physiological need for nutrients. It is the first coordinated muscular activity of the infant, which promotes the ingestion of maternal milk [1]. The urge for sucking exhibited in infants can be satisfied through nutritive sucking, including breast- and bottle-feeding, whereby the infant obtains essential nutrients, or through non-nutritive sucking on objects such as fingers and pacifiers, which are often used to calm down and comfort infants [2].

Many health benefits have been attributed to breastfeeding [3] including the promotion of adequate craniofacial development [4]. Whereas sucking at the breast requires intense facial and masticatory muscles activity, bottle-feeding and pacifier use promote different functional stimuli, which may impair the development and the strength of stomatognathic structures [5,6]. Furthermore, deleterious oral habits have been associated with orofacial dysfunction and higher impacts on oral health-related quality of life in children and adolescents [7].

The prevalence of non-nutritive sucking habits (NNSH) is quite variable and depends on several factors, such as age, gender, duration of breastfeeding, parents' educational level and number of siblings [2,8]. A birth cohort study showed that 48,9% of infants at age 1 developed a pacifier-sucking habit [9]. Among preschool children, a prevalence of 40.2% of nonnutritive sucking habits was obtained in a cross-sectional study; of these, 27.7% were pacifier-sucking [10].

Mastication is a sensory-motor activity responsible for the preparation of food for swallowing [11]. Although the evidence suggests a negative impact of bottle and pacifier use on the development of the stomatognathic system [12-14], the implications of these habits on the development of the masticatory function were less explored; moreover, it is unclear if the habit cessation is able to overcome the damage. Only one study evaluated the influence of breastfeeding duration and presence of sucking habits on masticatory parameters, showing that the use of pacifiers for more than 6 months and bottle-feeding for over 1 year were associated with lower scores on masticatory function between 3-5 years [15]. However, the protocol used to assess chewing in this study has not been validated for this age group, and the broader age range of the sample, disregarding the possibility of improving oral function with age, weakens their findings.

During chewing, the fragmentation and humidification of food occurs simultaneously with the perception of taste and texture of the food, influencing the chewing process [11]. In the initial process of taste perception, taste substances are dissolved in the salivary fluid layer to reach and stimulate taste receptors [16]. Taste sensitivity has been referred as one of the main drivers of food acceptance in children, and plays an essential role in eating behavior [17]. Understanding taste sensitivity in children can help in encouraging a healthier diet and, consequently, improved health outcomes in adulthood.

Masticatory ability and taste perception can affect food selection, conditioning the consumption of certain foods according to their relative hardness and flavour content, respectively. In addition, the digestion process appears to be associated to how well the food is masticated and poor chewing may result in decreased nutrient absorption [18]. Thus, considering that children are in an accelerated process of development, early assessment of the masticatory apparatus seems to be of great importance. In this sense, the objective of this study was to investigate the influence of pacifier habit cessation on the development of masticatory behavior and taste sensitivity in preschool children. The hypothesis tested was that masticatory behavior and taste sensitivity differ between children who have or do not have a pacifier habit, and that cessation would promote an improvement in taste and masticatory function development comparable to the children who do not have the habit.

Methods

Study design

The data were collected prospectively. Children born in 2013 or 2014 were evaluated in their kindergartens from the year they turned four to the year they turned five years-old. Evaluations were performed in three moments: at baseline, 6 months and 1 year of follow-up. This was a controlled clinical trial with a two-arm parallel design, registered in the Brazilian Clinical Trials Registry (ReBEC; http://www.ensaiosclinicos.gov.br/), protocol no. RBR-728MJ2. Ethical approval was obtained by the Research Ethics Committee of the School of Dentistry of Piracicaba, University of Campinas, under Protocol No. 1.712.802. Written parental consent was necessary to enroll in the study, as well as an assent by the child (assent term).

Sample

Children selected from seven public kindergartens in the municipality of Piracicaba, state of Sao Paulo (Brazil) composed the sample of this study. After anamnesis and clinical examination, and based on inclusion and exclusion criteria, a convenience sample of 148 eligible children was initially selected. A total of 47 children from the pacifier group dropped out of the study either because they could not interrupt the NNSH, because the parents were not willing to collaborate, or because the child was excessively attached to the NNSH. Of the 52 volunteers who were willing to remove the habit, 24 volunteers dropped out of the study because they changed preschool institutions or moved out of the city during the assessments. In the control group, 17 participants dropped out of the study because they moved to a different preschool during the study or did not cooperate with the evaluations. A flowchart shows the number of participants throughout the study and the final sample in each group (Figure 1).

The exclusion criteria were as follows: presence of dental caries (cavities), dental anomalies of number or shape and crossbite; history of oral or facial injuries; history or presence of orthodontic or speech therapy treatment; report or diagnosis of respiratory and / or food allergies; presence of systemic or local disorder that may compromise the masticatory system (e.g., neurological disorders, epilepsy, cerebral palsy, among others); use of medications that may directly or indirectly interfere with muscle activity, such as antihistamines, sedatives, syrups, homeopathy, or central nervous system depressant drugs; patients who did not collaborate with data collection. The inclusion criteria were the presence of an established primary dentition, and for the pacifier group the criterion was the presence of NNSH (pacifier). To be included in the control group, bottle-feeding and/or NNSH should not be present, and children with a history of NNSH were only admitted if that persisted at maximum until 2 years of age. The control group was also composed of children with normal occlusion.

The sample size was calculated considering the results found by Pires et al. (2012), in which a significant difference was found in the masticatory function score between children aged 3-5 years who used a pacifier for more than 6 months and controls without a NNSH (mean score = 10.1 ± 3.48 x 7.00 ± 3.15 for controls). Considering a power of the test = 0.80 and alpha level of 0.05, a minimum of 20 children per group was needed.

Anamnesis

The child's parents or caregiver were interviewed to obtain information regarding personal data, medical and dental history, nutritive and NNSH that composed the inclusion/exclusion criteria. More specifically, information about sucking habits was collected, including questions related to the frequency, duration, intensity and existence of other habits associated with suction, with regard to the pacifier and / or bottle in the mouth, the size of the orifice, the type of nipple, pacifier and brand.

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During the interview with the parents, the characteristics of foods consumed the day before were assessed using the "Guidelines for evaluation of food consumption markers in Primary Health Care", an instrument developed by the Ministry of Health (Brazil, 2015) to assess food consumption of children from 2 to 9 years old [19]. The questionnaire was designed to examine the consumption of sugary and processed foods (fruit, vegetables, beans, sausages, sweetened beverages, instant noodles and crackers, as well as the ingestion of sweets and sandwich cookies).

Clinical examination

One trained examiner, Dentist and Specialist in Orthodontics (KGOS), wearing personal protective equipment, performed the clinical oral examination in a brightly lit kindergarten room, using mirror, probe to exclude participants with cavities and/or crossbite, which are known to have an influence on masticatory parameters.

The decayed, missing and filled teeth (dmft) index [20] was applied by one examiner (KGOS) to assess the caries experience (WHO, 2013), while morphological occlusion were evaluated to identify the inclusion and exclusion criteria according to the criteria proposed by Baume (1950) [21] and Foster & Hamilton (1969) [22]. Overjet was determined by measuring in millimeters the distance between the buccal surface of the most projected maxillary incisor to the buccal surface of the corresponding mandibular incisor and later classified as normal (≤2 mm) or increased (>2 mm). The overbite was considered to be normal when the upper incisors overlapped the lower incisors by 2mm. Anterior open bite was present when the incisal edges of the lower primary central incisors were below the level of the incisal edges of the upper primary central incisors in centric occlusion. Posterior crossbite was defined as occlusion of the buccal cusps of the upper primary molars or the tip of the upper primary canine lingual to the cusps of the lower primary molars or the tip of the lower primary canine, respectively.

Body weight was measured to the nearest 0.1 kg with the use of a digital floor scale (Mondial Ellegance BL-03, Brazil). Standing height was obtained to the nearest 0.1 cm using a portable stadiometer (CESCORF, Brazil). Nutritional status was then evaluated using the Z-scores for BMI according to World Health Organization standards (WHO, 2013) [20].

Clarification Method for Removing the Pacifier Habit

The pacifier group was submitted to the Method of Clarification and positive reinforcement, which consists of explanations about the possible clinical changes that the sucking habit could induce, followed by strategies to reinforce the desired behavior [23-25]. This method has the advantage of being simple and feasible in the kindergarten setting, not requiring the child to attend to a dental / speech clinic visit. Parents or guardians were made aware of the importance of their integration in the context of the problem and, as an illustration of the consequences, photographs and book pictures demonstrating the clinical changes that the pacifier and bottle sucking habits may produce in their children were shown. Parents were instructed not to interfere with the child's decision, positively reinforcing when the child presented the desired behavior (that is, decreasing the frequency of the habit, or its complete abandonment). The number of sessions focused on guidance and awareness of children and parents or guardians varied from 4 to 6.

Illustrated calendars were distributed for each day and night, in which the child was instructed to draw pictures or markings when not using pacifier. The child was encouraged to make many drawings and thus could exchange them for stickers [24]. With this instrument, it was possible to observe the decrease in the frequency use until habit discontinuation.

Masticatory and swallowing functions assessment

The individual chewing skills were evaluated through the Mastication Observation and Evaluation (MOE) instrument, previously developed and validated for young children [26,27]. During the test, children were instructed to chew a crispy biscuit (Tostines Cream Cracker, Nestlé®) in their habitual manner. The masticatory function was then recorded using a camera (Canon EOS Rebel T3I) by one examiner (KGOS), at a standardized distance (1 m) from the child, fixed on a tripod with focus on the face, neck and shoulders. During the recording, the participant remained sitting in a chair with backrest and the feet resting on the floor. Evaluations were performed at baseline, 6 months and one year of follow-up.

The evaluation of recordings was performed by one blind speech therapist (KSGNM), who was previously trained and calibrated to perform the examination. A second speech therapist, PhD in Oral Physiology, was invited to participate in the study to serve as a gold standard in the training process (DAP). The following aspects were assessed: a) tongue protrusion; b) lateral tongue movement; c) squashing or sucking movement; d) jaw movements; e) chewing time; f) food or saliva escape; g) number of swallows; h) rhythm and coordination. The response options per item varied from 1 to 4: scores 1 and 2 represent inappropriate oral motor movement and scores 3 and 4 represent appropriate oral motor movement. The difference between 1 and 2, and 3 and 4 is the degree of (in) appropriateness.

The swallowing function was assessed through the “habitual swallowing” domain of the “Orofacial Myofunctional Assessment” protocol (MBGR) [28]. This domain included the following aspects: lip and tongue posture, food escape, mentum and orbicular contraction, head movements, noise, coordination and presence of debris after swallowing. The higher the score achieved, the more altered the function.

The chewing time (in seconds) was measured from the moment food was placed in the mouth until the last swallow was taken or when the mandible remained in resting position for ≥ 2 seconds; the number of chewing cycles required was also assessed, being defined as an upward and downward movement of the chin.

Assessment of taste sensitivity

The detection threshold for sucrose and urea were determined using an adaptation of the methodology proposed by Visser et al. (2000), previously developed and applied to young children [29]. Thirteen concentrations of sucrose (1.5, 3.0, 6, 12, 18, 24, 30, 45, 60, 120, 180, 240 and 300 mmol/L) and 15 urea concentrations (3.75, 7.5, 15, 30, 60, 120, 180, 240, 300, 450, 600, 1200, 1800, 2400 and 3000 mmol/L) were prepared periodically and stored in a refrigerated environment.

The test was presented to the child in a playful way, using a story in which a "magician" made "magic potions". It was explained to the child that the magician fumbled and mixed all the solutions. The liquids were presented in small cups containing 3 ml of each solution and the child was challenged to help her figure out which cup held the magic potion.

One glass with the tastant solution and one glass with distilled water were presented simultaneously. At first, the child tried a 300 mmol/L solution of sucrose (more concentrated) and a glass of distilled water; if the child failed to differentiate the sucrose solution from the water, the instructions were repeated and the test restarted. If the child missed more than twice, the test stopped and was resumed on another day. Subsequently, the concentration of 240mmol/L sucrose was tested. To increase the speed of the procedure, the concentration was initially reduced by two concentrations at a time until the first incorrect response occurred. Subsequent increases or decreases in concentration were always one step at a time. The test was interrupted when two incorrect answers were given at the same concentration. The detection threshold was set to the first level above this concentration. If a child had reached the lowest concentration with two correct answers, the test was also stopped. The same steps were followed to the detection threshold for urea.

Assessment of Reliability

A pilot study was conducted before beginning of the data collection to verify the reproducibility of the measurements. For that, 18 children that were not part of the study sample were selected. The detection of sucrose threshold was assessed twice, with an interval of seven days between the two assessments. The evaluation of MOE protocol was performed by two speech therapists. For both, repetion of sucrose sensitivity and evaluation of MOE assessment, the intraclass correlation coefficient was used.

Statistical analysis

Data were statistically analyzed using the SPSS 24.0 software (IBM Corp., NY, EUA) by one of the authors (PMC, Applied Statistics Spec), considering an alpha level of 5%.

Exploratory analysis consisted of means and standard deviation, medians and percentages (Chi-squared test). Normality was checked using the Shapiro-Wilk test; non-normal distribution variables were transformed using the natural logarithm, when necessary. No data imputation or elimination was performed.

Two-way mixed model ANOVA was used to test the effects of the within-subjects factor (time: baseline, 6 months and 1 year) and the between-subjects factor (group: control and pacifier) and the interaction between these factors in the observed variance of MOE and swallowing scores, chewing time and number of chewing cycles (considered as dependent variables). The effect size (partial Eta squared) and the power of the test for each model were also obtained. The results of the Box's test, Mauchley's sphericity test and Levene's equality of variances test were evaluated as assumptions; when necessary, the Huynh-Feldt correction was applied. Outliers were considered when the studentized residual was greater than ±3 SD.

Results

The intra-examiner reproducibility assessed during the pilot study ranged from good (MOE score; ICC = 0.7) to excellent (sucrose threshold; ICC = 0.9) [30].

The total sample who attended all the sessions comprised 60 children with a mean age of 48.2 months (SD 4.1), which were divided into two groups: a 'pacifier group', consisting of 28 children with NNSH (pacifier), including 24 children with open bite and 19 with overjet, and a 'control group' including 32 children without current sucking habits and with normal occlusion. The frequency of open bite in the pacifier group was 85.7% at baseline (mean = 2.4 mm), 7.1% (1.5 mm) at the 6-month follow-up and 3.5% at the 1-year follow-up. We also observed the presence of increased overjet in 67.8% of children in the pacifier group at baseline (mean = 4.35 mm), 42.8% at 1-year follow-up (mean = 3.5 mm) and 21.4% of children at 1-year follow-up (mean = 3.2 mm). The mean age (SD) of both groups at baseline was 48.2 months (±4.1).

In the control group, only 28% of children were exclusively breast-fed, the others were bottle-fed in their first months of life (Table 1). All children included in the pacifier group had a history of bottle-feeding, and the habit was present in 57.1% of the children in the beginning of the study. Recommendations for the cessation of bottle-feeding were delivered to the parents /caregivers, but despite that, 7% were still bottle-fed after 1-year of follow-up.

The description of food consumption and nutritional characteristics of the clinical groups are also found in Table 1. The median number of meals/day and the reported percentage consumption of fruit, vegetables, candies and processed food in the day before did not vary between groups ($p>0.05$).

Table 2 shows the difference between the control and pacifier groups for masticatory behavior and taste sensitivities at baseline and after pacifier removal (at 6 months and 1-year of follow-up). The analysis showed a significant difference in MOE scores between groups at all times evaluated, being higher for the control group ($p = 0.002$). This means that the control group presented more appropriate oral motor movement during mastication at all times evaluated, when compared with the pacifier group. It also increased over time for both groups (Figure 2), but the pacifier group did not reach the control group's score even after pacifier removal. On average, "squashing or sucking movement" was the behavior that contributed most to the lower scores achieved by the pacifier group, while "rhythm and coordination" were the aspects that most improved after cessation of the habit. At 1-year follow-up, 31.2% of the children from the control group reached a maximum score of 32 points (better performance), while this percentage was only 17.8% in the pacifier group.

The swallowing score significantly differed between the groups at baseline and 6 months; in both groups, this score decreased over time, and within 1 year both groups were no longer significantly different ($p>0.05$) (Table 2/ Figure 3). On average, "mentum contraction" was the aspect that contributed most to the worse performance presented by the pacifier group throughout the study. At 1-year follow-up, 4 of 32 children of the control group reached zero score (best performance), while 1 of the 28 children in the pacifier group achieved this score.

The chewing time (Figure 4) and the number of chewing cycles (Figure 5) were not different between groups in any of the evaluated moments, but the scores of both groups decreased over time ("time effect", $p<0.001$).

The sucrose threshold was significantly different between the two groups at baseline, being the sucrose sensitivity in control group significantly greater than in the pacifier group at the beginning of the study. In addition, sucrose sensitivity changed over time, increasing for the pacifier group and decreasing for the control group; at 1-year follow-up, the values were no longer significantly different (Table 2/ Figure 6). Bitter sensitivity did not show significant differences over time nor did it differ between the groups.

Discussion

The main finding of this study is that preschool children with a pacifier habit showed important changes in masticatory function that did not self-correct even 1 year after removing the sucking habit, highlighting the need for prevention and habit interruption as early as possible. In addition, the results emphasize the importance of a close monitoring of the development of masticatory function in young children with NNSH. Myofunctional and occlusal changes resulting from prolonged sucking habits are possibly the main factors responsible for the significantly lower MOE scores in the pacifier group in all evaluations. Some authors speculate that the lack of equilibrium in the oral cavity of children with sucking habits is due to a lower position of the tongue in the anterior part of the mouth floor, which in turn may disturb the dynamic balance between the tongue, cheeks and lips [31,32]. This incorrect tongue position on the mouth contributes to the development of malocclusion in the primary dentition.

In the present study, mastication was evaluated by observing the chewing ability of solid foods [26,27]; mastication can also be measured by other objective parameters such as the maximum voluntary bite force, which is related to the integrity of the chewing muscles and is considered an important tool for assessing the functional state of the masticatory system [33]. Electromyography and kinematics have also been used to assess how muscle activity and jaw movements develop and progress with age [34,35]. Such methods, although interesting from the developmental perspective, do not consider the dynamic aspect of the masticatory function and the influence of behavioral aspects, different textures of foods and difficulties. In this sense, our study expands the existing knowledge about the masticatory development, evaluating other important aspects such as lip closure, loss of food or saliva, rhythmic and coordination, posture, among others in children who use pacifier and also in control ones.

Interestingly, an improvement in mastication function was observed in both groups throughout the study, possibly due to a maturation of the orofacial muscles, what is expected for this age group [36]. However, even one year after cessation of the NNSH, children from the pacifier group did not reach scores similar to those in the control group. This finding suggests an immaturity of masticatory function in children with sucking habits, as seen in the frequent “squashing or sucking movement” behavior during mastication, which did not resolve after the pacifier was removed. The development of feeding skills occurs simultaneously with the transition from munching to chewing, which is characterized by the change from up and down movements of the jaw during munching to diagonal and lateral movements to ultimately reach a circular rotation movement [37]. Rudimentary rotation movement is evident at 12 months of age, reaching maturity around 2–3 years, depending on the degree of exposure to a variety of food textures [35].

From a developmental physiology standpoint, the swallowing pattern matures from infantile to somatic type in most children from the ages 2 to 4 years, but finger or pacifier sucking, bottle-feeding, mouth breathing, and tongue sucking have the potential to slower maturation of the swallowing pattern [31]. In agreement with previous studies, our results demonstrated that children with NNSH show important impairments in the swallowing score at baseline and also after 6 months, but after 1 year of pacifier removal, children with former

NNSH showed a swallowing pattern comparable to that of the control group. A previous study emphasized that sucking behavior until 5 years of age may be responsible for the persistence of an atypical swallowing pattern at the ages 6 to 9 years, showing the importance of interrupting the habits in young children as this altered pattern can be perpetuated [32].

Although the chewing time and the number of chewing cycles did not differ significantly between the two groups, in both groups they decreased over time, showing an improvement in masticatory efficiency. Contrary to these findings, a previous study [38] did not find significant effects of age on the time spent to eat food with different textures (solid, purée and viscous food) in 4- to 5-year-old children. However, the authors observed that the measures were strongly affected by the texture of the food eaten. In the present study, we only tested one type of texture (biscuit). Additional research should investigate the impact of sucking habits on eating behavior of foods with other textures.

The assessment of taste sensitivity revealed that sweet sensitivity was significantly greater in the control group than in the pacifier group at baseline. This difference may be related to the prolonged pacifier use, which is suggested to interfere in the evaluation of taste sensitivity in this age group [39]. This difference can also be attributed, in part, to the frequency of bottle-feeding habit with sugary milk observed at baseline in the pacifier group (57.1%), and a consequent adaptation of the taste buds, compared to children in the control group who did not have nutritional sucking habits at 4 years of age. In addition, the possible effect of continuous contact between the silicone or latex nipples and the oral cavity has not yet been investigated but should be considered, as it represents a persistent texture stimulus in the mouth.

Sucrose sensitivity significantly changed over time, increasing in the pacifier group and being no longer different 6 months follow-up. Again, as most of these children had discontinued the bottle-feeding habit during the study, this change in taste sensitivity can be attributed to dietary changes. Regarding the urea detection threshold, we did not find developmental differences in sensitivity for bitter taste, as it was stable across all times evaluated for both groups. Similarly, a recent study also described the stability of bitterness sensitivity in children between four to six years old [39]. One possible explanation for this finding could be the less exposure to the bitter taste in the child's diet. At birth, infants exhibit innate predisposition to dislike bitter taste probably as an important protective sense [40-42] to avoid the ingestion of poison and toxins [43]. Because infants display facial expressions of dislike in response to bitter tastes, caregivers may hesitate to continue offering bitterness foods. As the child grows, rejection of bitter tasting substances is evident in their rejection of liquid formulations of medicine and of certain vegetables, thereby interfering with healthy food consumption and potentially limiting intake of important nutrients. However, accumulating evidence suggests that repeated exposures to a variety of flavors enhances acceptance to new foods during childhood [44, 45].

As bottle-feeding is believed to represent a harmful stimulus on the development of masticatory function [15], the present study also attempted to look at this confounding factor. To our knowledge, this is the first study to investigate the effects of prolonged NNSH and the impact of their removal on masticatory function and taste sensitivity in preschoolers using a prospective design. Careful considerations were taken using validated protocols for masticatory assessment and taste sensitivity that were specifically designed for preschoolers, which we propose to be the main strength of the study. In addition, as oral health may have a strong impact on mastication, it is advisable that the dental conditions should be similar in all groups when evaluating masticatory function [46], which was warranted in this study in terms of including children without dental cavities and crossbite. However, one limitation of the study is the higher degree of open bite and increased overjet in the pacifier group - which could have influenced the masticatory performance and is probably attributed to the respective NNSH.

The study also has some further limitations. As previously mentioned, masticatory function was assessed using only solid food; other textures should be examined for a better understanding of the development of chewing behavior. In addition, we only obtained food frequency data at the beginning of the study, being not possible to follow up dietary changes after sucking habits removal. Furthermore, the anamnesis concerning bottle-/breastfeeding may be subject to inaccuracies, especially in those children that did not live continuously with their biological parents/caregivers.

Conclusion

This study showed that the detection threshold for sucrose differed between preschool children with and without pacifier habit at the age of 48.2 months, and changes in masticatory behavior did not self-correct even after 1 year of pacifier removal, highlighting the importance of discontinuing sucking habits as early as possible. In addition, monitoring the development of masticatory function and taste sensitivity in children with sucking habits and considering the need for intervention seem advisable even in a very young age.

Figure 1. Flowchart of research participants and the final sample in each group.

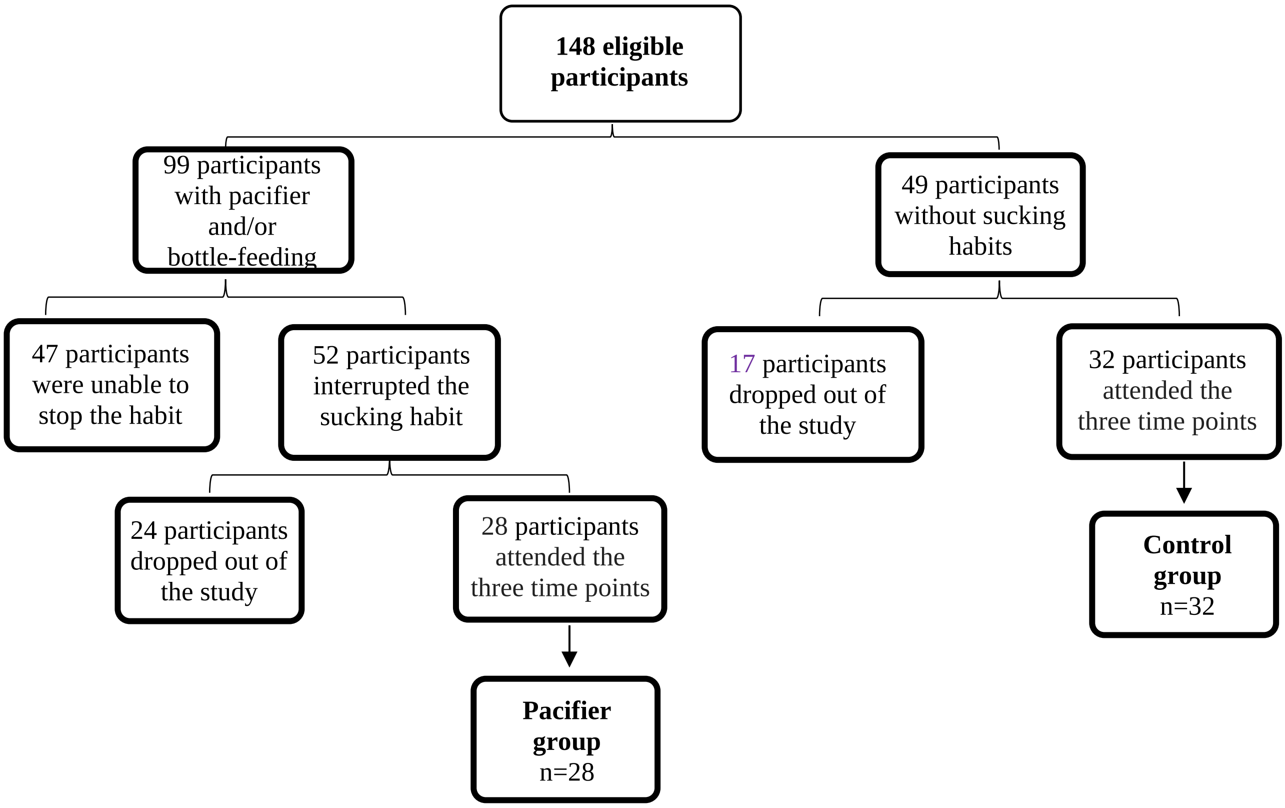

Figure 2. Time effect on Mastication Observation and Evaluation instrument (MOE) score for the control and pacifier groups ($p < 0.001$, Eta partial square=0.46, test power=1.00).

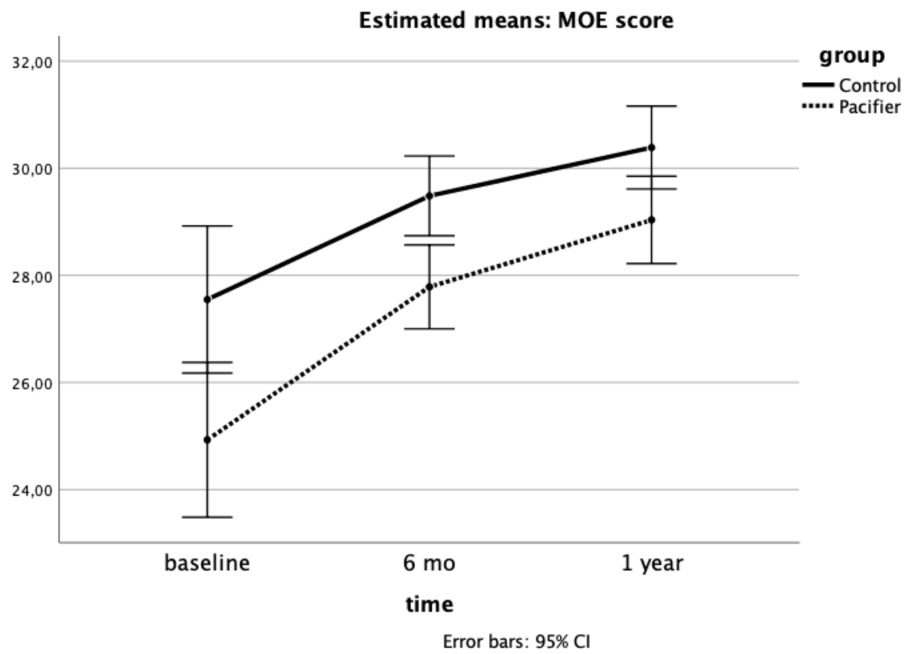

Figure 3. Interaction effect time*group on swallowing score for the control and pacifier group (Time effect p<0.001, Eta partial square=0.42, test power=1.00; interaction time*group effect p<0.001; Eta partial square=0.16, test power=0.99).

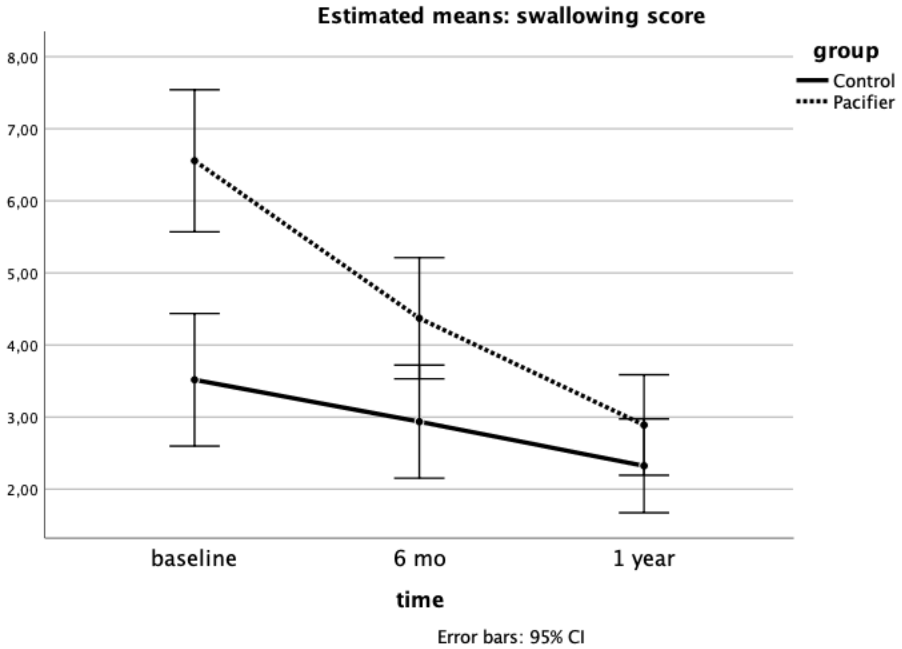

Figure 4. Time effect on chewing time for the control and pacifier group (Time effect p<0.001, Eta partial square=0.32, test power=1.00).

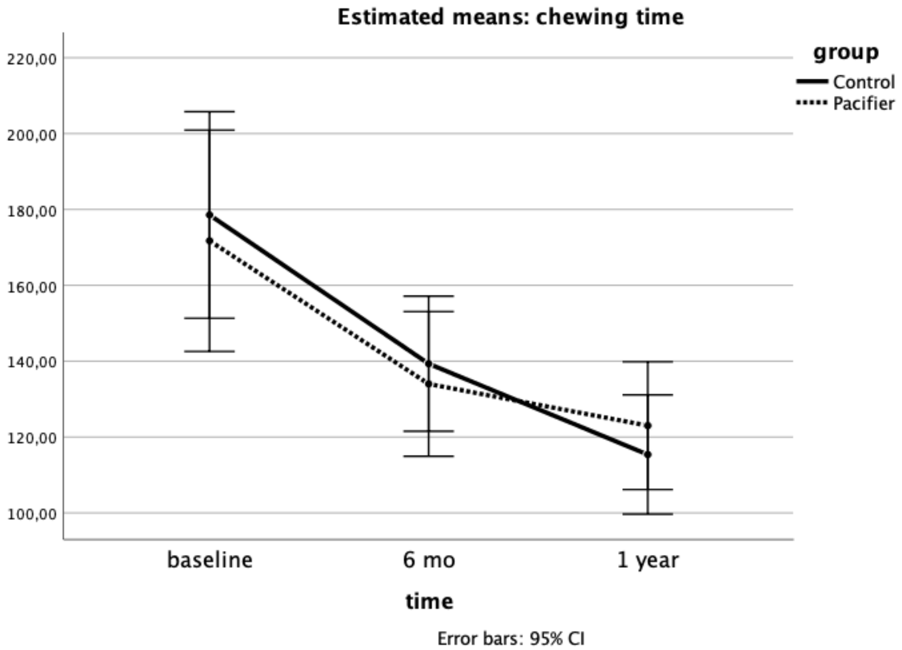

Figure 5. Time effect on the number of chewing cycles for the control and pacifier group (Time effect p=0.020, Eta partial square=0.07, test power=0.71).

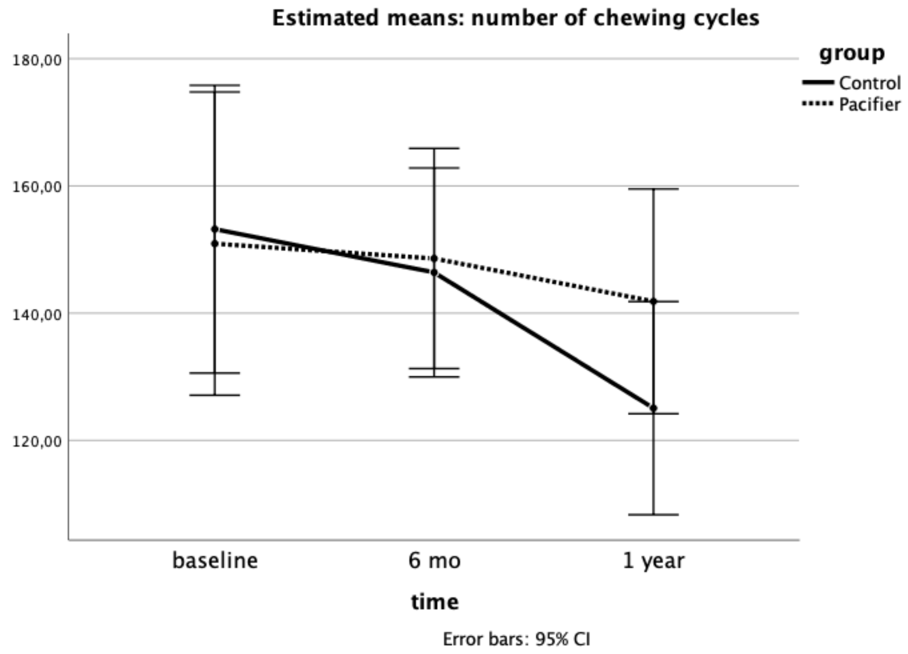

Figure 6. Interaction effect time*group on sucrose threshold for the control and pacifier group (Time effect p<0.001, Eta partial square=0.21, test power=0.99; interaction time*group effect p<0.001; Eta partial square=0.34, test power=1.00).

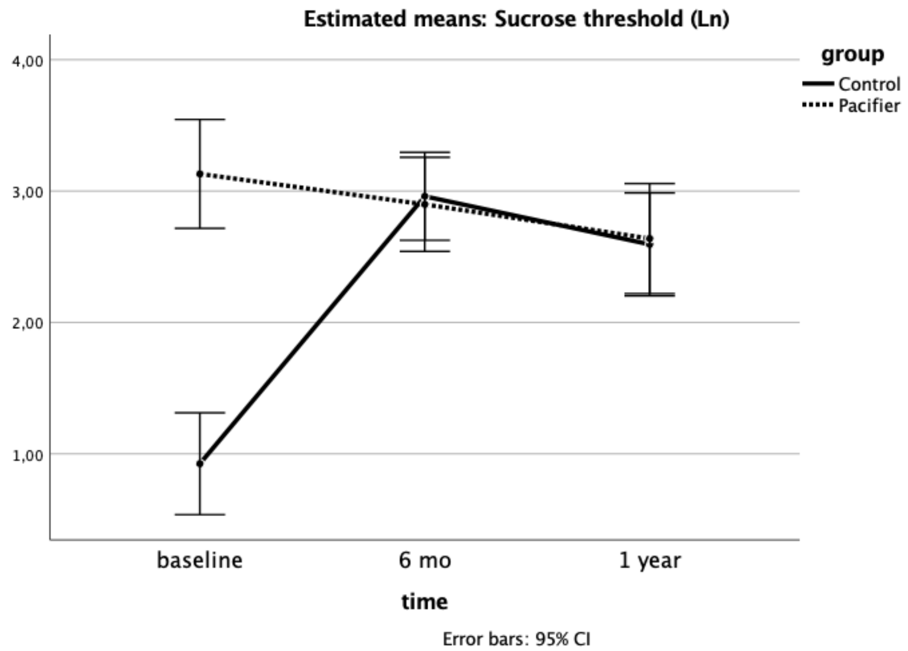

Table 1. Characteristics of the clinical groups at baseline

Group		**Control (n=32)**	**Pacifier (n=28)**
Sex	*f/m*	16/16	15/13
Age (months)	*Mean (SD)*	48.2 (4.1)	48.2 (4.1)
BMI (Kg/m^2)	*Mean (SD)*	15.7 (1.9)	15.8 (1.5)
Pacifier use	*History (%)*	19	100
	Median age of onset (months)	2	1
Bottle-feeding habit	*History (%)*	72	100
	Median age of onset (months)	9	2
	Number of bottles/day *(median)*	3	3
Food intake markers the day before			
Number of meals/day	*Median*	4	5
Beans	*%*	83	71
Fresh fruit	*%*	92	86
Vegetables	*%*	61	65
Hamburger and/or sausages	*%*	46	20
Sweetened drinks	*%*	79	80
Noodles, snacks, crackers	*%*	52	48
Cookies and candies	*%*	64	70

SD, standard deviation; BMI, body mass index.

Table 2. Interaction effect *time*group* on taste sensitivity and masticatory behavior: a Two-way Mixed Model

Group	Time	Masticatory function (MOE total score)	Swallowing score	Chewing time (seconds)	Number of chewing cycles*	Sucrose threshold (mmol/L)	Urea threshold (mmol/L)
		Mean (SD)					
Control (n=32)	baseline	27.4^{A} (2.9)	3.5^{A} (1.9)	175.0 (76.6)	152.6 (66.3)	6.2^{A} (10.5)	251.3 (242.7)
	6m	29.6^{C} (1.7)	2.9^{C} (1.8)	138.2 (49.4)	147.2 (45.3)	25.4 (16.3)	218.4 (142.5)
	1y	30.5^{E} (1.6)	2.3 (1.8)	116.2 (36.6)	124.7 (36.5)	19.1 (14.3)	163.4 (114.6)
Pacifier (n=28)	baseline	24.9^{B} (4.6)	6.6^{B} (2.9)	173.0 (76.4)	152.8 (58.1)	30.9^{B} (23.4)	226.1 (175.7)
	6m	27.8^{D} (2.4)	4.5^{D} (2.5)	132.9 (50.9)	148.5 (45.9)	27.0 (23.0)	170.4 (141.8)
	1y	29.0^{F} (2.6)	3.0 (1.9)	122.6 (52.3)	141.1 (55.6)	23.6 (16.7)	182.2 (137.2)
		p-value (*eta* partial^2/power of the test)					
Time effect		**<0.001 (0.46/1.00)**	**<0.001 (0.42/1.00)**	**<0.001 (0.32/1.00)**	**0.020 (0.07/0.71)**	**<0.001 (0.21/0.99)**	0.802 (0.01/0.08)
*Interaction effect time*group*		0.196 (0.03/0.34)	**<0.001 (0.16/0.99)**	0.607 (0.01/0.13)	0.345 (0.02/0.23)	**<0.001 (0.34/1.00)**	0.448 (0.01/0.19)

MOE, Mastication Observation and Evaluation instrument; SD, standard deviation
A ≠ B; C ≠ D; E ≠ F in the same column ($p<0.05$; One-way ANOVA)
* Number of chewing cycles required to eat a biscuit, being defined as an upward and downward movement of the chin.

Compliance with ethical standards

Conflict of interests The authors declare no conflict of interests.

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Ethical approval All procedures performed in children were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration.

Informed consent Informed consent was obtained from all parents of the participants included in the study.

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