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The Role of Nutrient Content in Diet in the Etiology of Dental Crowding

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The Role of Nutrient Content in Diet in the Etiology of Dental Crowding

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[redacted]

Dedication and Acknowledgments

I would like to dedicate this thesis to my parents, Darlene and Vipin Popat, and my siblings Poonam, Amal, and Jesal Popat. Thank you for supporting me from across the country and being there whenever I needed help. Also, thank you to my boyfriend, Samik Patel, for help in editing and formatting my many drafts of this thesis.

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ABSTRACT

BACKGROUND: Malocclusion affects almost two thirds of the American population. While malocclusion is unlikely to cause physical morbidity, crooked teeth pose a burden on quality of life and their treatment supplies a 10 billion-dollar per year orthodontic industry in the United States alone. Yet, it is a recent phenomenon since compared to recent anthropologic past the prevalence of malocclusion has sharply increased. However, there is currently little agreement among experts as to the reason for this change. Genetic theorists speculate that allelic redistributions are to blame. On the contrary, environmental theorists claim dental attrition, airway obstruction, functional habits, caries prevalence, diet consistency, and nutrient content each may contribute to the development of malocclusion. Surprisingly, few studies have been performed examining the influence of dietary nutrient content on the development of dental crowding. Insight gained may over time lead to improved preventative treatment options for malocclusion, increased stability of teeth, the promotion of oral health, and increased access to care.

OBJECTIVE: 1.) To provide a synthesis of studies addressing the contribution of nutrient content in diet and its influence on malocclusion. 2.) To design a study that would examine the effect of diet on the development of malocclusion while considering all potential confounders.

METHODS: A scoping systematic review was performed January of 2016 using PubMed and EMBASE databases. Studies were included if they met the following criteria: 1.) published in English and 2.) assessed diet and its possible relationship to malocclusion. Studies were excluded for the following reasons: 1.) Not published in English, 2.) Limited information on methods, 3.) focuses only on diet consistency and not nutrient content and other known etiologies of malocclusion as discussed already in the introduction or 4.) the full-text article is no longer in print.

RESULTS: After reviewing 1,689 articles found in the PubMed and EMBASE databases, 14 studies met the inclusion criteria and were chosen for analysis in the review. Eleven studies were cross-sectional and 3 were animal studies.

CONCLUSIONS: To date, no studies have measured nutrient content in diet and determined its role in the development of malocclusion while controlling for the following confounding factors: genetics, orofacial habits, attrition, caries, and diet consistency. Such studies are necessary to investigate the association between nutrient content in diet and malocclusion. Further high-quality studies are needed to gain etiological insight. Such knowledge could reduce the incidence and/or severity of dental crowding in the permanent dentition, improve access to public oral health, and reduce the burden of malocclusion on self-confidence and quality of life.

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BACKGROUND

About 2.8 million years ago, the earliest species of the modern human sharing the "homo" genus appeared on Earth. These earliest of humans species, known as *Homo habilis* [1] had a dentition close to the ideal Class I angle classification occlusion. However, it differed in a few subtle ways from our current norms. Most notably, malocclusion, which refers to the misalignment of teeth, did not then exist at appreciable levels[2]. This trend of straight teeth remained stable throughout the ascent of *Homo sapiens* from 250,000 years ago until 10,000 years ago, when teeth then began to shift[3,4].

Today, malocclusion effects almost two thirds of the American population[5]. Data from the National Health and Nutrition Examination Survey (NHANES III) show noticeable incisor irregularity in the majority of racial/ethnic groups within the US population, with only 35% of adults having well-aligned mandibular incisors not requiring any kind of orthodontic treatment[6]. There is currently no agreement among experts as to the etiology of this irregularity, with genetic theorists speculating that genetic hereditability and allelic redistributions are to blame, while environmental theorists claim dental attrition, airway obstruction, functional habits, caries prevalence, diet consistency, and nutrient content[7-45] play some role in the phenomenon.

To date, while few studies have been conducted to determine the genetic components of malocclusion, it is alleged that the time interval of the last 10,000 years is too brief for significant genetic variation to be the only cause of dental crowding. Findings thus far show that for Class I dental crowding, a reported SNP rs6504340 within the HOXB cluster is associated with delayed tooth eruption and occlusion irregularities. Additionally, genes EDA, XEDAR, and BMP2 were reported to have significant associations with dental crowding of more than 5mm[7].

These studies however were limited and performed on small sample sizes of participants with only Chinese genetic lineage.

Furthermore, a study by Bushchang et al, examined over 9,000 untreated Mexican American, Black, and White adults in the United States to determine genetic factors explaining individual differences in incisor irregularity. Their findings showed that ethnicity, the number of first and second molars, sex, and age combined are not associated with incisor irregularity and that the primary determinants of incisor irregularity remains to be determined[8]. Furthermore, past studies observing the transition of mild crowding to severe crowding within one to two generations of a family's lineage lead us to believe that genetics plays less of a role in the etiology of malocclusion than once assumed[9-11].

In contrast, Begg and others believe the etiology of dental crowding is due to environmental factors such as the lack of interproximal tooth attrition. *Homo Habilis* exhibited a flat plane of occlusion, in which cusps and deep fossa of newly erupted teeth were quickly worn down and interproximal surfaces of the dentition were flattened from the constant chewing of coarse and gritty foods [3,12-15]. The attrition of dentition led to a reduction in food traps and cusp fractures. The maxillary and mandibular arches were broad, impaction of third molars was minimal, and the jaws were aligned in an edge-to-edge occlusion[16]. It is believed that the broad, flat interproximal contacts within the dentition prevented the slipping of teeth buccally or lingually and allowed for stable positioning of teeth long-term. In present day, the dental occlusion of the human species has evolved to an occlusion where teeth maintain their cusps and fossas, interpromixal contacts are rounded, and third molars have less room to erupt into the arch[16-19], allowing for the considerable current prevalence of malocclusion.

However, recent studies have questioned the validity of Begg's theory[19-22]. Per Begg's studies of Australian aboriginal skulls, mandibular tooth reduction of over 10 millimeters and the phenomenon of mesial drift occurs to allow for all 32 teeth to fit into the dental arches. Prior to the eruption of permanent teeth, deciduous teeth were already worn to a significant extent but remained in interproximal contact through mesial drift[16]. In contrast, Corruccini points out that other studies also analyzing these same skulls found both severe and mild dental attrition with excellent occlusion and attrition measured at 20-40% less than calculated by Begg[9]. He claims, that if attrition and mesial migration of deciduous teeth were to occur in modern man, there would be impaction of the premolars. Additionally, also in contradiction to Begg, Fishman et al. found there was more dental crowding in dentitions with severe attrition when also examining skulls of Australian aborigines[19].

Other factors contributing to the development of malocclusion include functional habits and airway obstruction. Numerous reports have proven the effect of habits such as non-nutritive sucking, nail biting, tongue thrust, and mouth breathing interfere with the growth and development of the jaws and dentoalveoloar complex[5,23,24]. Chen et al. observed non- nutritive sucking habits are higher in those who have been weaned earlier[25]. The most important source of nutrition in the earlier years is breastmilk. Thus one may question, among individuals weaned early, whether malocclusion could be due to the early discontinuation of oral habit/ physical oral stimulation of sucking or to the nutritional content of the milk itself being removed from the individual at an earlier age. Individuals with chronic airway obstruction tend to develop a downward tongue posture, lowered mandibular posture, and a head that is tilted back to allow breathing from the mouth. This in turn leads to a constricted maxillary arch, open bite tendency and upright incisors[5].

With this shift away from a flattened plane of occlusion and well-aligned dentition 10,000 years ago came an increase in caries prevalence and malocclusion. This shift also coincided with the adoption of agriculture and a carbohydrate rich diet[26-28]. The agricultural lifestyle brought a greater reliance on plant foods and food preparation techniques. These processes reduced complex carbohydrates into simple sugars, the mono- and disaccharides, resulting in an increased caries rate[29]. More recently (~1850) the advent of industrially processed flour and sugar has further increased the rate of caries. Frequencies of carious lesions in archaeological populations range from 2.2–48.1% of teeth for agricultural populations, compared to the 0–14.3% for early hunter-gatherers[30,31]. Many recent studies have shown a positive correlation between early partial and full tooth loss due to childhood caries and dental crowding in the permanent dentition [32-34].

Structural factors to be considered in the etiology of malocclusion are the development of the jaws and dentoalveolar housing. Throughout hominid evolution, tooth size has been more influenced by genetic makeup compared to the housing of teeth which is more influenced by environmental factors[14]. Bone development is significantly influenced by nutritional and functional influencers, and enhanced bone development allows more room for teeth to fit within the maxillary and mandibular arches. Many animal studies have shown the importance of frequent mechanical function and increased masticatory forces for the development of robust jaw structures[35-37]. With the movement towards agriculture and industrialization of diet, masticatory forces and jaw sizes have decreased compared to previous ancestors[38-43].
In contrast, agricultural societies with non-industrialized, traditional diets maintain good occlusion and little to no dental crowding[10]. Admittedly, this is a multifactorial phenomenon. However, one can surmise that the lack of malocclusion in these societies may be due to diets rich in the specific nutrient building blocks necessary for bone growth and development, as well as elements that assist in tooth decay prevention. We know that key nutrients important for bone development and caries prevention include protein, fiber, calcium, potassium, magnesium, zinc and vitamins A, C, D, E and K2[44,45]. Furthermore, elemental factors such as the

buffering capacity of saliva, regulate caries balance through its influence on calcium's critical ability to neutralize the oral environment to minimize the de-mineralization of enamel and enhance its re-mineralization[46]. With the manifold etiologies of malocclusion, very few studies have been performed examining nutrient content in various diets and its influence on dental crowding. This is difficult to measure due to the multiple confounders of diet consistency, genetics, gestational, neonatal and postnatal nutrition also potentially influencing the development of the jaws and occlusion. A diet with key nutrients important for dentoalveolar bone growth and development at a young age could reduce the incidence and/or severity of dental crowding in the permanent dentition. Such knowledge could also empower orthodontists with more preventative modalities, improve access to public oral health, and reduce the burden of malocclusion on self-confidence and quality of life.

The first goal of this scoping systematic review is to provide a synthesis of the contribution of nutrient content in diet and its influence on malocclusion. The second goal is to design a study that would examine the role of diet on the development of malocclusion while considering all potential confounders.

METHODS

For this scoping systematic review, a search was performed January of 2016 using PubMed and EMBASE databases. A scoping systematic review is a preliminary assessment of potential size and scope of available research literature on a topic of interest. It aims to identify the nature and extent of research evidence that exists, as well as identify potential gaps within the literature. The goal of this scoping review was to provide a synthesis of the contribution of nutrient content in diet and its influence on malocclusion.

The following keyword search was used in PubMed and replicated in EMBASE: ((malocclusion or dental crowding or dental misalignment or class I crowding) AND (diet or food or nutrition or nutrient content or protein or fats or carbohydrates or vitamins or sugar or breastmilk or breastfeeding or neonatal or gestational or postnatal or formula)). Studies were included if they met the following criteria: 1.) Published in English or 2.) assessed diet and its possible relationship to malocclusion. Studies were excluded for the following reasons: 1.) Not published in English, 2.) Limited information on methods, 3.) focuses only on diet consistency and not nutrient content and other known etiologies of malocclusion as discussed already in the introduction or 4.) full-text is no longer available in print.

The initial search identified 1,135 in PubMed and 978 results in EMBASE. Bibliographies of studies which met inclusion criteria were reviewed for additional sources (N= 14). Duplicates to references in the PubMed database were excluded from other databases before identifying full-text articles to be included in the review (n = 1,689). After screening titles and abstracts of the articles, 1,663 records were excluded and 26 full-text articles were assessed for eligibility. The study designs and variables of interest were then reviewed to assess if malocclusion was measured and diet of the population was discussed. Twelve more articles were excluded for the following reasons: 1.) Limited information on methods (n=2), 2.) Focused only on diet consistency (n=2), 3.) Did not discuss the diet of the general population being studied (n=3), or 4.) did not measure malocclusion. Fourteen studies were included for assessment in the present systematic review. These results are presented in Figure 1 according to PRISMA guidelines.

For each of the studies included in the review, we assessed the study design used by the authors, the study population involved, the sampling strategy, and the sample size, which variables (exposure/risk factors and outcome variables) were measured, and how they were measured. The results for each study were reviewed, interpreted and compared to one another when feasible. In particular, the review of results for the various studies were organized by the following topics: genetics, oral habits, attrition and tooth size, caries, jaw size, nutrient consistency, and nutrient content.

RESULTS

Study Designs and Samples

After performing an extensive database search on PubMed and EMBASE, 14 articles were selected for further review based on our eligibility criteria (see Table 1). Of the 14 studies, 11 were designed as cross-sectional studies and 3 were designed as animal experimental studies. Of the cross-sectional studies, sample sizes ranged from 34 individuals to 2,200 individuals. Three studies separated the individuals into groups of rural versus urban, 3 studies separated the individuals by diet type, 2 studies compared boys and girls with a rural population, 1 study compared young adults in an urban population, 1 study compared individuals by different eras, and 1 study separated individuals by indigenous community and the resultant indigenous community of close relatives.

Sample sizes for the animal experimental studies ranged from 22 pigs to 108 pigs. All three studies compared undernourished pigs to normal or well-nourished pigs. One study further evaluated undernourished pigs after they were rehabilitated to a well-nourished diet.

Variables and Measures

All studies measured dental malocclusion of the individuals or animals being studied, as well as a measured or reported diet. Five out of the 11 human cross-sectional studies measured diet by utilizing a questionnaire. All the animal studies had detailed descriptions of diets fed.

Other major variables measured included dental status or DMFt (6 studies), periodontal status (4 studies), Angle's classification (5 studies), Dental Aesthetic Index (DAI) (3 studies), Treatment Priority Index (TPI) (1 study), attrition or tooth size (3 studies), orofacial habits (2 studies), arch width or length (3 studies), jaw size (3 studies) and socioeconomic status (2 studies).

The methods of all studies were assessed to evaluate potential confounders on the influence of nutrient content in diet and the development of malocclusion. The following confounders were considered: genetics (6 studies), orofacial habits (11 studies), caries status (8 studies), attrition of dentition (10 studies), consistency of diet (11 studies), or nutrient content of diet (11 studies). None of the 14 studies controlled for all six factors listed that may contribute to the development of malocclusion.

Study findings

a.) Genetics

In Lauc's study of inhabitants of Hvar Island and Normando et al.'s study of indigenous villages in Brazil, a significant amount of inbreeding was identified, however both study populations had traditional diets[47,48]. The Hvar population had more Class II malocclusions, deep bites, and midline shifts than the general Croatian population but not more than other modern urbanized groups. Similarly, the resultant indigenous village in Brazil had almost double the amount of malocclusion than the original indigenous village.

b.) Oral habits

In Abidoye et al.'s study comparing the oral health status of rural and suburban youths in Nigeria, Class I malocclusion was higher amongst the suburban children than rural children[49]. There was a statistically significant difference ($P<.05$) between the dietary habits of groups. The rural children mainly used chewing sticks to clean their teeth, whereas the suburban children used toothpaste and toothbrushes. A similar pattern was observed in Asawa et al.'s study of fisherman and non-fishermen oral health status[50]. The degree of malocclusion was higher among the non-fishermen compared to fishermen, and majority of the fishermen used chew sticks for cleaning their teeth whereas non-fishermen used toothbrushes. Other studies from this scoping review did not note habits, or controlled for any subjects with described habits by excluding those subjects from their results.

c.) Attrition and tooth size

In Lindsten et al.'s study comparing tooth dimensions and dental arch dimensions in children from various times and cultures, a smaller tooth size was found in the mixed dentition of children from 14th to 19th century skulls compared with contemporary children living in the same country of Norway[51].

In India, Sherfudhin compared the dietary habits and dentoalveolar characteristics of vegetarian and non-vegetarians[52]. With the exception of meat, the only significant difference in dietary patterns was the intake of sweets, of which non-vegetarians consumed at a higher rate. There was significantly more attrition in the vegetarians versus the non-vegetarians, and vegetarians had more crowding and less available space in the maxillary region.

In Tonge's animal experiment examining the dental tissues of rehabilitated animals which had undergone nutritional deficiency, it was found that the first premolars and first molars were as equally worn down as those in normal animals of the same age, however these animals had chewed significantly less food and less frequently[53]. Additionally, the nutritional deficiency in the experimental pigs caused retardation of growth and eruption in teeth, and may have reduced the size of the permanent dentition.

d.) Caries:

Lindsten et al.'s study captured the caries status of the second deciduous molars of mixed dentitions of Norwegian skulls from 14th and 19th centuries, Norwegian children born in the 1960s, native Norwegian Sami children born in the 1980s, and urban Norwegian children born in the 1980s[51]. The evaluation of partial tooth loss exhibited no difference between the ancient skulls and the experimental groups born in the 1980s. The Norwegian group born in the 1960s had the most frequent second deciduous molars with partial tooth loss and less arch space due to caries prevalence. The Sami group had greater caries prevalence than the ancient skulls and children born in 1980s, but less than children born in the 1960s. Finally, there was no significant difference in irregularity index among Sami children, Norwegian children born in the 1980s, and the Norwegian children born in the 1960s.

Corruccini et al. evaluated the oral health status of a single inbreeding population in Chandigarh, Punjab of different socioeconomic statuses[54]. In all socioeconomic groups the caries rates were low, however the level of dental crowding in the middle and high socioeconomic groups were statistically greater. Amongst middle and high socioeconomic groups processed foods dominate, while coarse millet and locally grown vegetables are staples amongst the lower socioeconomic groups. Raw sugar cane is also regularly chewed for enjoyment amongst the lower socioeconomic groups.

e.) Jaw size:

Wood et al. investigated the oral health status and malocclusions of 100 Alaskan Eskimo youths[55]. The Eskimo youths were found to be exposed to a modern diet and only one out of the 100 individuals had intact dentition. Sixty-four percent had Class I malocclusion and 18% had normal occlusion.

Corruccini et al. measured arch breadth and length of the maxilla and bigonial arch of the mandible of an inbred community in Kentucky[56]. Individuals in the study were separated into different groups based on their diet: Modern, semi-modern, semi-traditional, and traditional. No significant differences were found in the arch jaw measurements between all groups. However, occlusal variation increased with either increasing age or decreasing amounts of traditional components to the diet. Diet showed a stronger correlation to the treatment priority index than age.

Luke et al. describe the growth of the jaws and teeth in pigs which were maintained on a protein-deficient (PD) and calorie-deficient (CD) diets during the first and second years of life and then allowed unlimited food[57]. In animals who received CD and PD diets for 2 yrs., jaw sizes recovered to 96% of controls after rehab with liberal feeding. However, malposition of teeth did not correct itself. Tonge et al. performed a similar study on pigs with a CD diet until one year of age, and found resultant overcrowding, displacement and malocclusion of teeth[53]. The calorie deficient pigs were found to have not only insufficient cranial and facial growth, but also reduced facial height, palate length, palate width and small size of the mandibular ramus.

f.) Nutrient consistency:

As mentioned previously, Lindsten et al. studied the mixed dentitions of Norwegian skulls and children found no significant difference amongst the groups[58]. The Sami children were brought up traditionally and were introduced to harder foods early, often containing dried meat and harder bread than usually consumed by other children. In Corruccini et al.'s study on the rural community of Central Kentucky, those members of the community raised on softer and more cariogenic foods were more variable from the ideal in their occlusal patterns than those with diet of harder consistency[56].

In the animal studies performed on pigs by Tonge et al. and Luke et al., all pigs were fed specific diets with similar consistencies and varying nutrient content[57,59]. Though consistencies in the differing diets were held constant, the degree of malocclusion varied greatly between the nutrient complete and nutrient deficient pigs.

g.) Nutrient content:

Overall, in the studies by Abidoye et al., Asawa et al., Corruccini et al., and Chandra et al., where the occlusion of rural populations with traditional diets were compared to urban populations with modern diets, it was found that the rural populations had less dental crowding[49,50,60,61]. In Pujara et al.'s study on determining the effect of westernization on the oral health of college students of Udaipur City, Rajasthan, malocclusion showed a significant relationship with participants who preferred eating English food for snacks and dessert[62].

Tonge et al. and Luke et al. studied the effects of nutrient deficiency on the development of the jaws and dentition[53,57]. Nutrient deficient pigs were found to have increased malocclusion, a delay in the eruption of teeth, and a greater delay in growth of the jaws. Once the nutrient deficient pigs were rehabilitated to a normal diet, jaw sizes recovered to 96% of controls, however malocclusion did not correct itself.

DISCUSSION

Few studies have been performed analyzing the influence of nutrient content in diet on the development of malocclusion. For this project, a scoping review of fourteen studies was performed to provide a synthesis of this relationship. Each study measured malocclusion and either discussed the general diet of the study population, or measured the diet through a questionnaire. In order to gain insight into the relationship between nutrient content and malocclusion, information is needed for both macro- and micro-nutrient content of the diet. Of the 14 studies, only the 3 animal studies measured the exact nutrient content in diet fed to their animals and could describe the effects of their diet in the development of malocclusion. This is insufficient to draw strong conclusions from. The remaining 11 cross-sectional studies recognized the role of diet in the development of malocclusion but did not specifically measure the nutrient content in the diets of the individuals. Moreover, none could control for all confounding factors related to the development of malocclusion. However, these studies helped to elucidate the role diet may play in the development of malocclusion compared to other factors such as genetics, orofacial habits, attrition and tooth size, caries, jaw size, and nutrient consistency.

In order to understand the potential causes of malocclusion, one must attempt to parse out the signal from the noise of confounding variables. Of its many possible etiologies, the subject of genetics is often the first to be attributed as the cause of rising rates of malocclusion. In this analysis – in particular, the studies analyzing the malocclusions of highly inbred communities -- it was found that though these communities possessed traditional diets similar to their original village, the resultant, highly inbred community had a significantly higher percentage of malocclusion[47,63]. While this may be due to a variety of factors including the revelation of otherwise hidden recessive traits, the process of genetic inbreeding of close relatives is seen to alter occlusal development. In a situation in which there is a high degree of incestuous relationships within a population, genetics may play a greater role than nutrient content in diet on the development of malocclusion. It is unclear to what degree a community must be inbred in order to genetically affect dentoalveolar development. The implication of these findings points the importance for future studies to control for the level of inbreeding through genetic testing.

Studies within the scoping review did not note habits found within the populations. Alternatively, it is possible that studies which looked at habits were excluded from the results entirely due to not meeting the stated inclusion criteria. While not exactly a habit, per se, a couple of studies noted a difference in oral hygiene habits between some rural and urban populations. Rural populations used chewing sticks to clean teeth compared to urban individuals who were more likely to use toothbrushes and toothpaste to clean teeth[50,64]. Interestingly, the rural individuals were found to have significantly less dental crowding than urban individuals. Additionally, the diets between the rural and urban populations were notably different. Due to the insufficient design of these studies, it is unclear whether the habit of chewing sticks or nutrient content in diet plays a larger role in reduced malocclusion. Furthermore, no studies have been done analyzing the nutritive effects of breast milk on the growth and development of the jaws, alveolar housing, and dentition while controlling for non-nutritive sucking habits. This brings into question if individuals were weaned early, could malocclusion be due to the early discontinuation of the oral habit / physical oral stimulation of sucking, or to the nutritional content of the milk itself being removed from the individual at an earlier age? The question of habits vs nutrition and malocclusion is further analyzed later on in the discussion in relation to the effects of dietary content on attrition.

Another controversial topic on the etiology of dental crowding is the influence of attrition or small teeth in prevention of malocclusion. In Lindsten's study, a smaller tooth size was found in the mixed dentition of children from 14th to 19th century skulls compared with contemporary children living in the same country of Norway. The evaluation of the loss of tooth substance in the second deciduous molars showed no difference between the skulls and the Norwegian group born in the 1980s. However, the irregularity index of the four permanent mandibular incisors was significantly greater in the ancient skulls than in other groups[58]. Similarly, in Sherfudhin's study comparing the dietary habits and dentoalveolar characteristics of young adults with vegetarian and non-vegetarian diets, there was significantly more attrition in the vegetarians versus the non-vegetarians. However, the vegetarians had more crowding and less available space in the maxillary region compared to the non-vegetarians[52]. These findings reject the theory that increased attrition or smaller tooth size leads to a higher likelihood of developing a normal occlusion. Through these studies, it seems diet content may play a greater role in the prevention of malocclusion than attrition or small sized teeth.

Additionally, in rehabilitated animals which had undergone nutritional deficiency, their first premolars and molars were as much worn down as those in normal animals of the same age and who had chewed more food for a longer time. Thus, frequency of chewing may not contribute to the amount of wear that develops on teeth. In combining this phenomenon with the rural villagers who have less malocclusion than their urban counterparts and who chew sticks to clean their teeth, it could be the increased masticatory forces from the chewing sticks helping to stimulate alveolar bone development and reduce dental crowding rather than the chewing sticks wear on the dentition slimming the teeth to reduce dental crowding. Just like tensile stresses from muscles inserting onto bone can stimulate bone growth in that area, it is possible that the alveolar housing surrounding teeth could be stimulated to grow from the tensile stresses of masticatory forces during a critical window of development. However, none of the included studies were designed in a way to differentiate between diet content and attrition vs alveolar bone growth throughout the growth of the viscerocranial complex.

In addition to losing tooth structure through attrition, the presence of dental caries is thought to lead to early partial or full tooth loss and an increase in dental malocclusion. In Lindsten et al.'s study, the Sami group had greater caries prevalence than the 14th and 19th century skulls and children born in 1980s but less than the children born in the 1960s. The 1960s group had less arch space due to significantly greater caries prevalence. Conversely, although the 1960s group had less arch space, there was no significant difference in irregularity index between this group and the Sami children born in the 1980s and the Norwegian children born in the 1980s[58]. Assuming the dietary habits between all groups were different, and knowing the loss of tooth structure and arch space did not greatly affect irregularity index, dietary habits could conceivably play a larger role in dental crowding than caries prevalence. Similarly, the Corruccini et al. study evaluating the oral health status of a single inbreeding population in Chandigarh, Punjab shows possible support for this theory[54]. The results showed that in all socioeconomic groups, while the caries rates were low and showed no statistical differences between each other, the level of dental crowding in the middle and high socioeconomic groups were statistically greater than in the low socioeconomic group. In the medium and high socioeconomic groups, processed foods predominated, while in the same location's low socioeconomic groups, coarse millet and locally grown vegetables are staples with raw sugar cane regularly chewed for enjoyment. This corroborates the findings previously discussed that the habit of chewing sticks or raw sugar cane has been shown to be correlated with reduced malocclusion compared to urban populations lacking in this habitual activity.

However, none of the studies included in this review have controlled for this habit in their studies.

With bone development of the jaws and alveolar housing being significantly influenced by nutritional and masticatory factors, it is important to differentiate if the jaws and alveolar housing are effected with equal or different magnitudes by a lack in either masticatory or nutritional factors. Wood et al. examined 100 Alaskan Eskimo youths who were exposed to a modern diet. The previous generations of these youths were raised on traditional diets and were found to have slight to no malocclusion[55]. In Wood's study, 64% percent had class I malocclusion and 18% had normal occlusion. The higher percentage of class I malocclusion in this study indicates that the basic jaw relationship of the primitive Eskimo has been retained, however the increase in malocclusion indicates the alveolar housing or dental development may have been affected. Furthermore, Corruccini et al. found no significant differences in the arch jaw measurements between all diet groups within an inbred community of individuals with different levels of traditional diets[56]. However, occlusal variation increased with decreasing amounts of traditional components to the diet. Additionally, animal studies observing the jaw and dental development of nutrient deficient pigs found overcrowding, displacement and malocclusion of teeth, as well as insufficient cranial facial growth[53,57,59]. After rehabilitating the nutrient deficient pigs with liberal feeding, jaw sizes recovered to 96% of controls yet malposition of teeth did not correct itself. These results demonstrate that diet may affect alveolar housing size more than jaw size and that malocclusion is established by early malnutrition and persists even after nutritional status and jaw sizes are restored.

As previously mentioned, bone development is not only influenced by nutritional factors but masticatory factors as well. The effect of diet consistency on the development of malocclusion is difficult to separate from the effect of nutrient content in diet on the development of malocclusion. However, it is difficult for studies to differentiate between nutritional content, diet hardness and consistency, attrition, and their effects on the development of malocclusion. In regard to diet consistency and hardness, the previously discussed Lindsten's study deserves additional attention. Irregularity indices were compared between the Sami, Norwegians born in the 1980s and the Norwegians born in the 1960s. There was no significant difference amongst the groups even though the Sami diet differed from the other groups. The Sami children were brought up traditionally and were introduced to harder foods early[58]. If we compare this to Corruccini et al.'s study on the rural community of Central Kentucky, we find that members of the community raised on softer and more cariogenic foods were more variable from the ideal in their occlusal patterns than those with diets of harder consistency[56]. Nutrient content in diet of these studies were not measured and it is unclear whether diet consistency or nutrient content played a greater role in the development of malocclusion. It is easier to attempt to control for these direct relationships within animal studies. In the animal studies performed on pigs by Tonge et al. and Luke et al., all pigs were fed specific diets with similar consistencies and varying nutrient contents. Though consistencies in the differing diets were held constant, the degree of malocclusion varied greatly between the nutrient complete and nutrient deficient pigs[53,57,59]. These studies show a greater influence of nutrient content in diet on malocclusion than the consistency of diet itself.

As previously mentioned, none of the cross-sectional studies in this scoping review measured nutrient content in a population's diet. Outside author Weston Price was unique in that he specifically measured nutrient content in diet of isolated populations around the world. In these populations, dentitions were nearly completely free from dental caries and had little to no dental crowding. Per figures used by the United States Department of Labor, the minimum adult requirements for calcium and phosphorus are 0.68 grams and 1.32 grams per day. The primitive populations examined by Price provided a nutrient content containing at least four times the minimum suggested requirements. Additionally, diets were rich in fat soluble vitamins which aid in nutrient absorption. Price's studies proved immunity to caries can be restored by changing nutrient content in diet to meet the minimal nutrient requirements[10]. If nutrient content in diets are kept to minimal requirements, early caries and partial or full tooth loss may be prevented and thus prevent an increased risk in malocclusion. Furthermore, maintenance of a nutrient rich diet could aid in craniofacial and dentoalveolar growth and provide greater support for the developing dentition.

CONCLUSION

To date, no studies have measured nutrient content in diet and determined its influence in the development of malocclusion while controlling for the following confounding factors: genetics, orofacial habits, attrition, caries, and diet consistency. Based on the results of this scoping systematic review on the role of diet in the development of malocclusion, it was found that in populations of high inbreeding, genetics may play a greater role than nutrient content in the development of malocclusion. With regards to the habit of chewing sticks, it is unclear whether the chewing habit or the nutrient content plays a larger role in the prevention of dental crowding.

Diet has a greater influence on malocclusion than attrition or small sized teeth, and malocclusion is established by early malnutrition, persisting even after nutritional status and jaw sizes are restored. No studies have analyzed the nutritive effects of breastmilk on the growth and development of the jaws, alveolar housing, and dentition while controlling for non-nutritive sucking habits. In animal studies, with control for diet consistency, there seems to be a greater influence by nutrient content in diet on malocclusion than the consistency of diet itself. Furthermore, diet may affect alveolar size more than jaw size and if nutrient content is kept to minimal suggested requirements, early caries and partial or full tooth loss may be prevented and thus the increased risk of malocclusion.

From the conclusions drawn from this review, future studies can be designed which examine the role of nutrient content in dental crowding, while controlling for confounding factors of malocclusion. A case control study comparing individuals within a population with low severity of malocclusion (ages 13-16) to individuals with moderate-to-severe malocclusion would be surveyed on their mothers’ diet during pregnancy and lactation, their duration of breastfeeding, and their dietary habits prior to the eruption of his/her permanent dentition.

However, the issue with this type of design is that diet measured at adolescence would be used as a proxy for diet consumed in early childhood when the teeth and jaw are developing. Alternatively, parents could be asked to remember their child's diet 10 years earlier, but this would lead to an even bigger measurement error or bias with parents of children with malocclusion have selected memories of their children's diet. Similarly, a cross-sectional study is a way to perform an exploratory study at relatively modest cost of both time and funds. However, this type of study design captures only one time point of an individual's diet. Furthermore, it is unable to provide insight into causality since diet would be measured simultaneously with malocclusion at an adolescent age at which point malocclusion has already developed. Due to the need of capturing diet throughout growth and development and prior to the development of malocclusion, a more appropriate design would be a cohort study following a population of individuals and their diets from infancy to age 14 and compare the diets of individuals who develop malocclusion over time with those who do not. Such knowledge could reduce the incidence and/or severity of dental crowding in the permanent dentition, empower orthodontists with more effective treatment and preventative modalities, improve access to public oral health, and reduce the burden of malocclusion on self-confidence and quality of life.

FIGURES

Figure 1: PRISMA Flowchart of Results

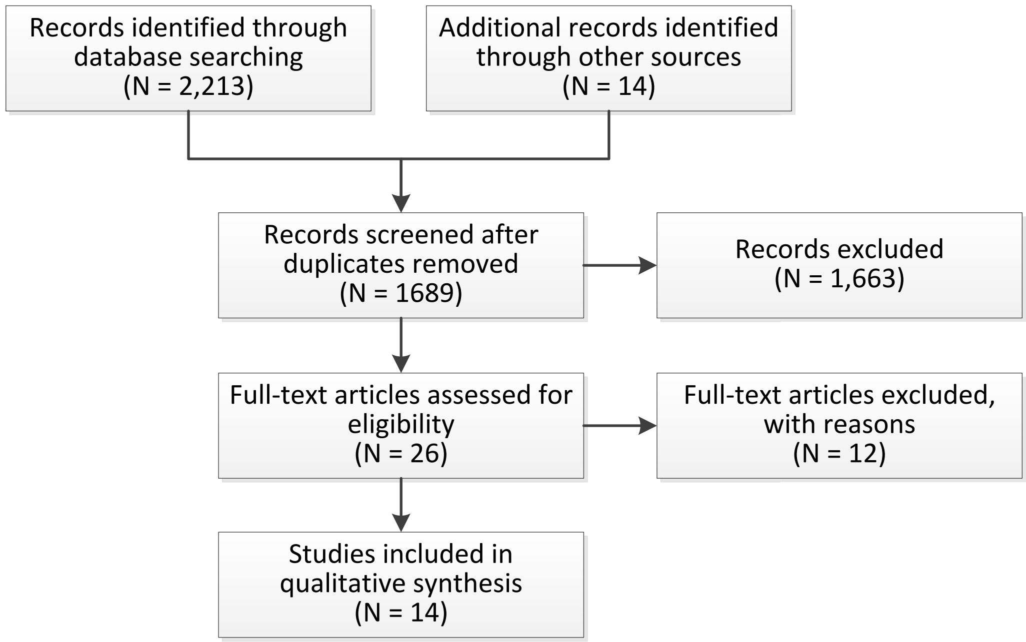

Figure 2: Summary of Results

Author, Year, Location	Study Objective	Study Design	Sample Size	Variables, Measures, and Confounders	Key Findings / Conclusions
Abidoye et al., 1993, Nigeria[49]	To determine the dietary practice and assess dental status of 12-year-old primary school children in rural and suburban communities of Ile-Ife and Imesi-Ile.	Cross-Sectional Study	N = 297 rural children N = 297 suburban children	- Diet habits by 24hr food frequency recall questionnaire - Caries status by DMF - Periodontal status by gingival index - Oral hygiene status with Greene and Vermillion index - Traumatic dental injuries by Garcia-Godoy method - Dentofacial anomalies by Bjork method <u>Confounders:</u> - Malocclusion and diet were measured however no statistical analyses were performed between the two. - Other confounders not controlled for in study: attrition, habits, diet consistency, genetics, and nutrient content in diet	- Class I malocclusion was higher among the suburban school children - There was a statistically significant difference (P<.05) between the dietary habits of both the suburban and rural children for all meals in the two towns - Majority of suburban children used toothbrushes and toothpaste whereas more rural used chewing stick - Rural had almost perfect teeth "finding is probably due to the well-developed jaws found among the rural population and the tendency to approximal attrition of teeth"
Asawa et al., 2014, India[50]	To assess and compare the oral health status of fishermen and non-fishermen population of Kutch District, Gujarat, India.	Cross-Sectional Study	N = 1100 Fishermen N = 1100 Non-fishermen	- Community Periodontal Index (CPI), - Decayed Missing Filled Teeth (DMFT) - Dental Aesthetic Index (DAI) scores taken <u>Confounders:</u> - Diet was discussed but not measured - Other confounders not controlled for in study: diet consistency, nutrient content in diet, attrition, and genetics	- Degree of malocclusion was higher among the non-fishermen (40.5%) compared to fishermen (27.1%) - 62.9% of fishermen and 59.5% of non-fishermen had no abnormality or minor malocclusion - Majority (43.1%) of the fishermen used chew sticks for cleaning their teeth while majority of non-fishermen (48%) used toothbrushes. - Fishermen population showed significantly greater proportion of subjects with dental caries (82.6%) as compared to non-fishermen population (44.6%)
Lindsten et al., 2003, Norway[58]	To compare tooth dimensions and dental arch dimensions in children from various times and cultures.	Cross-Sectional Study	4 groups (mixed dentition): N = 48 skulls from the 14th to 19th centuries N =39 boys and 34 girls of Sami origin born in the 1980s living in Norway N = 31 boys and	- Mesio-distal (MD) tooth sizes measured - Arch chord length measured - Available arch space measured - Irregularity of 4 mandibular incisors measured - DMFt measured <u>Confounders:</u> - Diet was discussed but not	- A smaller tooth size was found in the mixed dentition of children from the 14th to 19th century skulls compared with contemporary children living in the same country - Smaller MD tooth sizes in the medieval samples cannot be explained by attrition as a major factor -. The 1960s group had less arch space due to the

			30 girls born in the 1960s in Norway N = 32 boys and 26 girls born in 1980s in Norway	measured - Other confounders not controlled for in study: diet consistency, nutrient content in diet, habits and genetics	greater caries prevalence - The evaluation of the loss of tooth substance in the second deciduous molars showed no difference between the skulls and the Norwegian group born in the 1980s. The Norwegian group born in the 1960s had the most second deciduous molars with loss of tooth substance because of caries. The Sami group was intermediate. - The Sami children were brought up traditionally and introduced to harder foods early. - The irregularity index for the four permanent mandibular incisors was significantly greater in the skulls than the other groups. There was no significant difference among the groups born in the 1980s and 1960s.
Lauc T., 2003, Croatia[47]	(1) to evaluate the prevalence of some occlusal traits and malocclusions among the population of Hvar Island, Croatia; (2) to determine whether some of the occlusal traits are more prevalent compared with the general Croatian population; (3) to evaluate the age group that is critical in the development of occlusion; and (4) to provide the basis for future studies of genetic influence on the prevalence and progression of malocclusions.	Cross-Sectional Study	N = 126 males N = 98 females from all schools in Hvar island, Croatia (age 7-14)	The following were measured from dental models of subjects: - Molar relationship - Overjet - Anterior Crossbite - Midline relationship - Overbite - Crowding <u>Confounders:</u> - Diet was discussed but not measured - Other confounders not controlled for in study: diet consistency, nutrient content in diet, habits, caries and attrition	- Inhabitants of Hvar with a high rate of inbreeding and traditional diet have more Class II malocclusions, deep bites, and midline shifts than the general Croatian population - Since the lack of chewing stress in this population cannot be considered as the main explanation for the above phenomenon, the genetic influence on the development of these occlusal traits in the inbred populations should be further investigated.

Normando et al., 2011, Brazil[65]	To examine genetic and tooth wear influences on occlusal variation in a split indigenous population. The Arara-Iriri people are descendants of a single couple expelled from a larger village. In the resultant village, Arara-Laranjal, expansion occurred through the mating of close relatives, resulting in marked genetic cohesion with substantial genetic differences.	Cross-Sectional Study	N = 130 individuals from the Laranjal tribe N= 46 individuals from the Iriri village (ages 2-22) Tooth wear was examined only for individuals with permanent dentition (n = 81)	Clinical examination measuring: - Stage of dentition as deciduous, mixed or permanent - Early loss of deciduous and/or permanent teeth - Dental anomalies -Right and left molar sagittal relationship in the permanent dentition classified as Class I, Class II or III - Malocclusion - Tooth wear <u>Confounders:</u> - Diet was discussed but not measured - Other confounders not controlled for in study: diet consistency, nutrient content in diet, habits, and caries	- With traditionality of diet and similar tooth wear patterns in both groups being similar, malocclusion was found in 1/3rd of the original population and almost doubled for the resultant, incestuous, indigenous population
Pujara et al., 2016, India[66]	To determine the effect of westernization on the oral health of college students of Udaipur City, Rajasthan.	Cross-Sectional Study	N = 800 college students on Udaipr City, Rajastan (ages 19-25)	- A structured 24-item questionnaire designed to ascertain the effect of westernization on oral health. - Clinical exam measuring: Community Periodontal Index (CPI), Dentition status and treatment needs, and Dental Aesthetic Index (DAI) scores <u>Confounders:</u> - Other confounders not controlled for in study: diet consistency, nutrient content in diet, habits, genetics and attrition	- About 54%, 33%, and 34% of subjects preferred English food for lunch, snacks and desserts - Malocclusion showed a significant relationship with participants who preferred eating English food for snacks and dessert
Sherfudhin et al., 1996, India[52]	To investigate dietary habits, caries prevalence, and dentoalveolar characteristics, including tooth wear, in vegetarian and non-vegetarian young adult southeast Indians.	Cross-Sectional Study	N = 30 Indian vegetarians matched with N = 25 Indian non-vegetarians (ages 17-27)	- Questionnaire including detailed analysis of type of food and drink intake, number of daily meals, and between meal sweets - Habits recorded - Clinical examination measuring: DMFT - Examination of models measuring: number of malpositioned teeth, malocclusion class, overbite, overjet, and tooth wear <u>Confounders:</u>	- Except that the vegetarians ate no meat, the only significant difference found in dietary patterns between the groups was the intake of sweets. Non-vegetarians consumed sweets more often. - There was significantly more tooth wear in vegetarian vs non-vegetarian - Vegetarians had more crowding and less available space in the maxillary region - Increased occlusal wear

				- Other confounders not controlled for in study: diet consistency, nutrient content in diet, and genetics	and increased malocclusion in the vegetarians contradict the hypothesis that greater occlusal wear is associated with less crowding
Wood et al., 1971, Alaska[55]	To examine if the lack of children maintaining their routine dental checkups and oral hygiene has resulted in an increased rate of malocclusion.	Cross-Sectional Study	N = 100 Eskimo children in Alaska (ages 11-20yrs)	- Questionnaire asking to fill out approximate types and amounts of food eaten and dental care received Clinical examination measuring: - Malocclusion - Absence of teeth - Intercanine width - Intermolar width <u>Confounders:</u> - Other confounders not controlled for in study: diet consistency, nutrient content in diet, attrition, caries, and habits	- This population has been exposed to modern diet with poor oral hygiene care - Only one child had intact dentition - 64 % had Class I malocclusion, 18% normal - The higher percentage of class I malocclusion in this study suggests the basic jaw relationship of the primitive Eskimo has been retained
Chandra et al., 2013, India[61]	To compare the overall dental aesthetic index scores between rural and urban areas, males and females, and to correlate dental aesthetic index score with fluoride concentration in drinking water.	Cross-Sectional Study	N = 1268 in the district of Nalgonda in India (rural and urban 15-year olds)	- Questionnaire examining: age, type of diet (vegetarian/mixed), how often sweets are consumed, habits, oral hygiene habits, and socioeconomic status - Fluoride concentration among areas of study population - Clinical exam measuring: DAI score <u>Confounders:</u> - Other confounders not controlled for in study: diet consistency, nutrient content in diet, attrition, caries, and genetics	- 15.5% of the study subjects in rural areas and 22.9% in urban areas had a DAI score of more than 25. - The prevalence and severity of malocclusion was more among the urban population than the rural. - 27.6% of the study subjects in areas with below optimal fluoride concentration, 13.6% in the areas having optimal fluoride concentration, and 8.8% in the areas with above optimal fluoride concentration had DAI score of more than 25.
Corruccini et al., 1981, Kentucky[67]	To describe occlusal variation in the rural community of Central Kentucky along the guidelines of the Treatment Priority Index (with some modification), to correlate occlusal	Cross-Sectional Study	N = 12 under 25 N = 22 over 25	- Subjects separated by diet: Modern, semi-modern, semi-traditional, traditional - Dental models measured for: Treatment Priority Index (TPI) - Bigonial mandibular breadth measured <u>Confounders:</u> - Other confounders not controlled for in study: diet consistency, nutrient content in diet, attrition, caries, and habits	- Occlusal variation increases with either increasing age or decreasing amounts of traditional components to the diet - Arch jaw measurements show no significant correlations - Diet shows a stronger correlation to TPI than age - Members of the community raised on “softer and more cariogenic foods” are more variable from ideal occlusion

	variation with dietary and age variation and to correlate occlusal variation with a measure of inbreeding.				
Corruccini et al., 1983, India[54]	To survey occlusal variation in Chandigarh youths in relation to various environmental and social characteristics, especially diet.	Cross-Sectional Study	N= 78 High SEG N= 116 Middle SEG N= 71 Low SEG (ages 14-16)	- Clinical exam measuring: tooth emergence, periodontal status, DMFS, Angle's classification, crowding, overjet, overbite, crossbite, arch length - Socio-economic group(SEG): Low, med, High <u>Confounders:</u> - Diet was discussed but not directly measured - Other confounders not controlled for in study: diet consistency, nutrient content in diet, attrition, and habits	- Punjabi population taken from a single inbreeding population - Caries rates were low in all SEGs - Visual inspection of attrition showed to be minimal - Crowding: 3.7% in high SEG, 4.3% In Med SEG, 2.8% in Low SEG - Statistically significant crowding is present between low and high/med SEG - In urban Chandigarh, processed foods are more common, while in the country coarse millet and locally grown vegetables are staples. Raw sugar cane is regularly chewed rurally
Tonge et al., 1973[53]	To work out the steps of development that lead to normal occlusion in normal pigs, and secondly to find out why malocclusion develops in undernourished pigs.	Animal Study	N = 70 'large white' pigs: (37 control, 40 severe calorie restriction)	- Fed pigs normal and calorie deficient diet, observed occlusion until they were 5 years old - Food intake of pigs accurately measured <u>Confounders:</u> - Other confounders not controlled for in study: Habits, caries, and attrition	- Tonge(1965) performed study on same pigs with calorie-deficient diet until they were one year old and found resultant overcrowding, displacement and malocclusion - Severe undernutrition produced: "a.) A delay in the formation and eruption of the whole dentition b.) A greater delay in the development and growth of the jaws c.) Overcrowding of the developing and standing teeth and particularly of the permanent molars"
Luke et al., 1981[57]	To describe the growth of the jaws and teeth in pigs which were maintained on a protein-deficient (PD) and calorie-deficient (CD) diets during the first year of life and then allowed	Animal Study	White pigs: N = 39 controls N = 14 PD until 1yr old N =10 CD until 1yr old N = 24 rehabilitated PD N = 16 Rehabilitated CD N = 5 CD until 2yrs old then	- Animals fed specified diets - Animals killed at varying ages - Jaw and tooth size measurements performed - Crowding of molar teeth measured <u>Confounders:</u> - Other confounders not controlled for in study: Habits, caries, and attrition	- In animals who received CD and PD diets for 2 yrs., jaw sizes recovered to 96% of controls after rehab with copious feeding - The crowns of 3rd molars are only restored to 75% of controls after rehab - Malposition of teeth does not correct itself - Growth is delayed in pigs with malnourished diets

	unlimited food. Some pigs are also described which were fed CD diet for 2 years after birth and then rehabilitated.		rehabilitated		
Tonge et al., 1965[59]	To examine the dental tissues of rehabilitated animals which had undergone nutritional deficiency.	Animal Study	N = 12 undernourished pigs N = 6 normal pigs N = 4 well-nourished pigs	- Pigs killed at different time points - Selected measurements were made of the neurocranium, facial skeleton and teeth. - Attrition and malocclusion recorded <u>Confounders:</u> - Other confounders not controlled for in study: Habits and caries	-" Significant facts which have been brought out are that the degree of undernutrition to which these pigs were subjected (a) altered the shape of the mandible and the line of eruption of the incisor teeth, (b) retarded the growth and eruption and may have reduced the size of the permanent dentition, (c) delayed the absorption of the deciduous dentition, (d) produced malocclusion of the teeth, (e) created a discrepancy between the size of the teeth and the space available for them in the jaw, (f) interfered with the development of the enamel, dentine and cementum, (g) allowed the teeth and their surroundings to undergo repair, to move and react to stresses by alterations in the cementum, alveolar bone and periodontal membrane." - Teeth were affected less than other tissues. Only those parts of the teeth which were being formed during the period of undernutrition were affected. - The first premolars and the first molars which erupted during the time of the experiment were as much worn down as those in normal animals of the same age, although they had not taken part in chewing so much food and had been functional for a shorter time. - The malocclusion and crowding of the teeth in the

					jaws was due to insufficient cranial and facial growth, reduced facial height, reduced palate length and palate width and the small size of the mandibular ramus.

Part II:

Study Protocol: Determining the Role of Nutrient Content in Diet in the Development of Malocclusion

Abstract:

Malocclusion affects almost two thirds of the American population. While malocclusion is unlikely to cause physical morbidity, crooked teeth pose a burden on quality of life and their treatment supplies a 10 billion-dollar per year orthodontic industry in the United States alone. Yet, it is a recent phenomenon since compared to recent anthropologic past the prevalence of malocclusion has sharply increased. However, there is currently little agreement among experts as to the reason for this change. Genetic theorists speculate that allelic redistributions are to blame. On the contrary, environmental theorists claim dental attrition, airway obstruction, functional habits, caries prevalence, diet consistency, and nutrient content each may contribute to the development of malocclusion. Industrialized, Westernized diets of the developed world today are composed of highly refined foods. Through the refining process, nutrient content is decreased and sugar and caloric content are increased. On the other hand, traditional, agricultural diets are composed of fresh and unrefined foods which are rich in nutrient content and low in sugar and calorie content. It is thought that a diet low in nutrient content from early childhood to the development of the permanent dentition affects the development of the dentoalveolar housing of the jaws, thus reducing the space available for permanent dentition to erupt and creating dental crowding. Surprisingly, few studies have been performed examining the influence of dietary nutrient content on dental crowding. Insight gained may over time lead to improved preventative treatment options for malocclusion, increased stability of teeth, the promotion of oral health, and increased access to care. The aims of this cohort study are to 1.) Determine if a low nutrient content diet in childhood is positively associated with the development of dental crowding and 2.) If longer duration of breastfeeding is associated with a lower prevalence of dental crowding. Three hundred study

participants from San Francisco and 300 participants from Tzucacab,Yucatan, Mexico will be evaluated at ages 3, 10 and 14 with participation of their mothers. Inclusion criteria for participants include individuals who are 3 years of age, participants mother is able to be present and able to participate, and participant is expected to stay in their city throughout the duration of the study. Diet composition, orofacial habits, and breastfeeding duration will be documented through detailed questionnaires. Dental crowding and caries prevalence will be measured by clinical examination with use of ICON and DMFS indices.

1. BACKGROUND/RATIONALE

1.1 Introduction

Today, two thirds of the American population are affected by dental malocclusion. Dental crowding affects the quality of life of individuals by reducing their self-esteem and confidence. Moreover, it can make it difficult to maintain good oral hygiene. This can lead to expensive dental treatment to correct their malocclusion. From the conclusions drawn from the systematic review on "The Role of Nutrient Content in Diet in the Etiology of Dental Crowding", the etiology of malocclusion is currently thought to be multifactorial since it seems to be influenced by genetics, orofacial habits, caries, diet consistency, and attrition. Additionally, the development of malocclusion can be influenced by dietary habits early in life during the growth and development of the craniofacial structures and dentoalveolar complex. A diet with key nutrients important for dentoalveolar bone growth and development at a young age could reduce the incidence and/or severity of dental crowding in the permanent dentition. This may seem counterintuitive since malocclusion has become more prevalent in the developed world where richer foods are more accessible. However, westernized diets contain heavily refined foods which are often lower in nutrient content and high in sugars, which can lead to caloric excess. Also, high levels of dietary fructose has been implicated in toxicities similar in effect to excessive alcohol. Additionally, the reduction in breastfeeding duration that has accompanied industrialization may also provide a key window for developmental "hit and run" injuries to arch length due to early nutrient deficit. To date, no studies have explored the influence of nutrient content in diet or breastfeeding duration on the development of malocclusion while controlling for relevant confounding factors such as genetic mixing, orofacial habits, attrition, caries, and diet consistency. Genetic mixing occurs when individuals from different ethnic and racial backgrounds begin interbreeding.

A cross-sectional study is a way to perform an exploratory study at relatively modest cost of both time and funds. However, this type of study design captures only one time point of an individual's diet. Furthermore, it is unable to provide insight into causality since diet would be measured simultaneously with malocclusion at an adolescent age at which point malocclusion has already developed. Due to the need of capturing diet throughout growth and development and prior to the development of malocclusion, a more appropriate design would be a cohort study following a population of individuals and their diets from infancy to age 14 and compare the diets of individuals who develop malocclusion over time with those who do not. Genetic mixing, orofacial habits, caries, and diet consistency would all be controlled for through a variety of measures consisting of questionnaires and clinical exams.

1.2 Relevant Literature and Data

The scoping systematic review on the role of diet in the development of malocclusion revealed disagreement in the literature over the relative weight of etiologic factors contributing to the development of malocclusion. For instance, in populations of high inbreeding, genetics may play a greater role than nutrient content in the development of malocclusion. Alternatively, in regard to the habit of chewing sticks, it is unclear which of the chewing habit or the nutrient content plays a larger role in the development of dental crowding.

The review revealed that diet seems to have a greater influence on malocclusion than attrition. It also suggested that malocclusion is established by early malnutrition, persisting even after nutritional status and jaw sizes are restored. The role of early nutritional status is still unclear since no studies have analyzed the nutritive effects of breastmilk on the growth and development of the jaws, alveolar housing, and dentition while controlling for non-nutritive sucking habits. In animal studies, with control for diet consistency, there seems to be a greater influence of the nutrient content in diet on malocclusion than the consistency or “hardness” of diet itself. Furthermore, diet may affect alveolar size more than jaw size. Poor nutrition consequentially favors the development of dental crowding even in presence of normal sized jaws.

Previous literature has used validated indices of Decayed-Missing-Filled Surfaces (DMFS) and Index of Complexity, Outcome and Need (ICON) to measure caries prevalence and severity of malocclusion. Food frequency questionnaires, such as ASA24, and the Dental Quality Index-International (DQI-I) have been used to measure nutrient content in diet and quality of diet. Age, gender, habits, breastfeeding duration and other demographics have been measured through additional questions included in questionnaires.

1.3 Compliance Statement

This study will be conducted in full accordance to all applicable UCSF Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46, and the HIPAA Privacy Rule. Any episode of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent and assent and will report unexpected problems in accordance with The UCSF Committee on Human Research Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

2. STUDY OBJECTIVES

2.1 Primary Aim

The primary aim of this study is to determine if a low nutrient content diet in childhood is associated with a higher incidence of crowding of the permanent dentition

2.2 Secondary Aim

The secondary aim of this study is to determine if longer duration of breastfeeding is associated with a lower incidence of crowding of the permanent dentition.

3. STUDY DESIGN AND POPULATION

We propose to conduct a prospective cohort study among two target populations: 1) a rural population of Mexican children; and 2) an urban population of children in California. Accessible populations for this study consist of 1) children born and living in Tzucacab,Yucatan, Mexico (representing a racially homogenous, indigenous, agricultural population) and 2) children born and living in California (representing a heterogenous, westernized population). Participants will be selected by convenience sampling from 1) local census data on households with children of or under 3 years of age and 2) Children attending preschool (including Head Start) in San Francisco

The study is planned to take place from April 2018- April 2031. The first two years will be allowed for recruitment of 300 study participants in each cohort whom will continue to be followed from age 3 to ages 10, and 14.

With the collaboration of the local dental school of Merida, the local division of public health will be contacted to obtain a list of the local daycares and school programs of Tzucacab, Yucatan, Mexico. The dental students of Merida will contact these centers to gather all participants and their mothers to be screened, with permission, at the local elementary school. The San Francisco study population will be recruited from daycare centers and Head Start programs throughout the city. The San Francisco Unified School district will be contacted to obtain a list of the local daycare and Head Start programs in the city. Parents in San Francisco will be contacted via phone through the daycare center or Head Start program to meet for a scheduled screening.

Inclusion Criteria:

a.) Children who are 3 years of age

b.) Participant mother is able to be present and able to participate

c.) Participant is not expecting to move from city within the duration of the study

Exclusion Criteria:

a.) Craniofacial anomalies

b.) Patient has undergone orthodontic treatment within duration of study

c.) Children who's parents are migrant workers

4. OVERVIEW OF PROCEDURES

After obtaining authorization to conduct studies by the specific households, we will meet with the individuals and their mothers. The study and its voluntary nature will be explained thoroughly. Interested mothers of participants will be given a consent form and a questionnaire for them to complete for their child regarding dietary and orofacial habits. The mother will be required to complete the questionnaires at ages 3 and 10 years of age. At age 14, the participant may complete the questionnaires by his or herself and will undergo a clinical oral examination. At age 10, the participant will also undergo a brief DMFS examination to follow progress of caries and missing teeth in the permanent dentition.

The dental examination at age 14 will be comprised of an extra oral assessment of the participant's smile (esthetic component of the ICON) and an intraoral examination of the teeth and occlusion (DMFS and ICON index). At age 10, a DMFS exam will be included. No radiographs will be exposed and no treatment will be provided. The exam will include a soft tissue examination (extra-oral head and neck, intra-oral), tooth count and status (number of teeth present including partially erupted teeth, visible third molars, and visible dental root tips or fragments), and coronal dental caries (DMFS Index for permanent teeth). Each participant will also complete a food frequency questionnaire. Diet questions in the questionnaire will include: foods consumed within the last 24 hours, portion size of each food, and times of when foods were consumed. An additional questionnaire will include patient demographics, orofacial habits, and breastfeeding duration.

All exam data will be directly entered into an electronic form on a secured laptop and transmitted to UCSF and/or Local University electronically. The data will be entered interactively using a Microsoft access database. These MS Access files will then be sent, securely in an electronic format and then converted to MS Excel.

5.0 VARIABLES AND MEASURES

5.1 Predictor and Outcome Variables

Predictor variables:

Diet composition: The ASA24 food frequency questionnaire will assess food intake for study participants. ASA24 was created by the National Cancer Institute for clinicians to utilize as a tool to collect 24-hour food records from patients and receive complete nutrient analysis of their diet in a timely manner. The ASA24 tool can be used for epidemiologic, interventional, behavioral or clinical research[68]. Timing, quality, and quantity of all food consumed will be recorded and nutritional data will be calculated from published nutrient tables. Nutrient content data obtained from the ASA24 database will then be used to determine diet quality with use of the Diet Quality Index- International (DQI-I) index. The DQI-I score of the individual will be measured categorically as low (0 to ≤45), average (45 to ≤ 60), or high (>60).

Breastfeeding Duration, orofacial habits and demographics: An additional questionnaire will be used for the mother of the participant to assess breastfeeding duration and orofacial habits of participant from infancy to adolescence. Breastfeeding duration will be measured as a continuous variable.

Outcome variable

Severity of malocclusion ICON index: Malocclusion is a continuum ranging from an ideal occlusion to considerable deviation from normal, this includes measurement of dental crowding. For this study, the ICON index will be used. With the ICON Index, dental crowding will be categorized with a score of 0-5. A score of zero represents minimal to no crowding, a score of 5 represents severe crowding. Calibration exercises will be performed to 100% agreement using 10 casts from UCSF orthodontic clinic patients that are not participating in the study. Dr. Kjeld Aamodt has been trained and calibrated in using the ICON and will train and calibrate all the other participants in the study.

What is 5.2 Diet Quality Index- International variable

At each timepoint, the mother of the participant or participant will fill out a food frequency questionnaire known as, ASA24. From ASA24, we are able to obtain the following data which will then be used to determine the participants diet quality through use of the DQI index:

- Food intake per day (Kcal)
- Portion size of foods (grams)
- Frequency of food intake (occurrences/day)
- Nutrient content in foods (percentages)

Nutrient content of diet based on ASA24 will be measured as a continuous variable which will be categorized as low (0 to ≤45), average (45 to ≤ 60), or high (>60).

5.3 Demographic, Orofacial Habit, Breastfeeding Duration Questionnaire

- Gender- binary (male or female)
- Weight- continuous variable
- Height – continuous variable
- Present or past orofacial habit (thumbsucking, nail biting, pacifier use)- binary (yes/no)
- If yes, frequency of habit (hours/day)- continuous variable
- Duration participant was breastfed - continuous
- Population homogeneity defined by race/ethnicity of participant, parents and grandparents- categorical variable
- Chewing habits (chewing sticks, chewing gum)(hours/day)- continuous variable

5.4 Dental caries DMFS index

- Decayed, missing, and filled surfaces of teeth index- discrete variable (1-148)

5.5 ICON

- Amount of dental crowding in millimeters as score of 0-5- categorical variable

6. STATISTICAL CONSIDERATIONS

6.1 Primary and Secondary Endpoints

The primary objective is to determine if a low nutrient content diet (DQI <46) in childhood is associated with a higher incidence of dental crowding. The primary endpoint will be the difference in dental crowding between participants with a low nutrient content diet and those with high nutrient content diet.

The secondary objective is to determine if duration of breastfeeding is inversely associated with dental crowding. The secondary endpoint will be the difference in dental crowding between participants with short breastfeeding duration (under 12 months) and those with long breastfeeding duration (greater or equal to 12 months).

6.2 Measures to Avoid Bias

Calibration exercises will be performed to 100% agreement using 10 casts from UCSF orthodontic clinic patients that are not participating in the study. Dr. Kjeld Aamodt has been trained and calibrated in using the ICON, DMFS and ASA24 and will train and calibrate all the other participants in the study.

6.3 Sample Size and Power

If using survival analysis to compare an expected incidence of malocclusion among children with a lower nutrient diet in the US with an expected incidence of malocclusion of 50% among children in rural Mexico (thus a effect size of 20%), and assuming a normal distribution with a confidence level of 95% and power of 80%, we would need a sample size of 227 participants for each cohort to be able to detect a significant difference in malocclusion incidence between these groups. In order to account for attrition of participants, 300 participants for each cohort will be necessary to detect any significant differences between the groups in Mexico and in San Francisco.

7. STUDY ADMINISTRATION

7.1 Data Collection and Management

All exam data will be directly entered into an electronic form on a secured laptop and transmitted to UCSF and/or Local University electronically. The data will be entered interactively using a Microsoft access database. These MS Access files will then be sent, securely in an electronic format and then converted to MS Excel.

To protect potentially sensitive health information, randomly generated unique ID numbers for subject's questionnaire responses which are blind to the subject's observed oral health, malocclusion, and caries measurements will be used. Locked vessels for transportation and storage of paper forms with unique ID numbers will be used. All devices used will be passcode protected and encrypted.

7.2 Confidentiality

All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy and the Investigator and other site personnel will not use such data and records for any purpose other than conducting the study.

7.3 Regulatory and Ethical Consideration

7.3.1 Risk Assessment

Potential risk of participation will be explained to participants through the informed consent process. A small amount of school time will be interrupted for each subject which should not amount to more than 30 minutes per participant. Risks to individuals are slight, and involve mainly a small but potential loss of privacy. Information regarding results from the clinical exam will be given to the students to share with their parents in a sealed envelope to protect the students’ privacy. No information will be asked about legal documentation status. Potential Discomfort: able to tolerate mouth opening for 30 seconds or less for visualization of teeth using an intraoral dental mirror.

7.3.2 Potential Benefits of Study Participants

We will be providing services by dispensing toothbrushes, toothpaste, and oral exams which may aid in the participant's oral health and the participant's family's understanding of the oral health status of their child. We will send a form home to parents indicating if we recommend their child to be seen by an orthodontist or dentist. We feel that risks are minimal and justified.

7.3.3 Risk-Benefit Assessment

While risks to subjects may be a few and rare, they are real. However, we surmise that any ostensible detriment will be far outweighed by the benefits.

7.4 Informed Consent/ Assent and HIPAA Authorization

Consent, Assent, and Patient Bill of Rights Documents can be viewed in the attachments section of this application. The study and its volunteer nature will be fully explained to the participants. Interested participants will be asked to complete an informed consent. Our study team will return as many times as required to examine and interview all the participants eligible to participate at each household.

Informed Consent:

Informed consent in English or Spanish will be obtained from the parents/guardians for themselves and their children. Children will also provide assent. Parents will be free to decline participation for themselves or their children without consequences, and to decline to answer any specific questions on the questionnaire.

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