

**Plaque Acidogenicity Resulting from Beverages Consumed after
Sugary Cereal**

BY

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THESIS

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I would like to dedicate this thesis to my family. Particularly to my understanding and caring husband, Kshitij, who has always been very supportive and inspirational and to our precious kids, Rishi and Siddhant, who are the joy of our lives. It is also dedicated to my parents who believe in diligence, science, art, and the pursuit of academic excellence.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BMI	Body Mass Index
CDC	Centers for Disease and Control
COMA	Committee on Medical Aspects of Food
FL	Froot Loops
g	Gram(s)
GTF	Glucosyltransferase
hrs	Hours
IL	Illinois
IP	Insoluble Polysaccharides
IRB	Institutional Review Board
min	Minute(s)
ml	Milliliter(s)
NIH	National Institutes of Health
NIDCR	National Institute of Dental and Craniofacial Research
RTE	Ready To Eat
SD	Standard Deviation
UIC	University of Illinois at Chicago
U.S.	United States
WPI	World Precision Instruments

SUMMARY

The acidity of the oral cavity can be modulated by food consumption. One approach to evaluate the acidogenic potential of a food involves the determination of plaque pH following ingestion. Cereal, milk and fruit juice are the most commonly consumed breakfast foods in the United States. The acidogenic potential of cereal depends on its sugar content and retentiveness on the tooth surface; but it can be altered with the inherent properties of beverages consumed after cereal consumption.

The objective of this study was to examine the effect of whole milk, apple juice or water on dental plaque acidogenicity in adults after dry sugary cereal (Froot Loops) consumption. Twenty adults (18-64 years) participated in this randomized controlled crossover study. The test food groups were dry Froot Loops (FL) cereal, and dry FL cereal followed by milk, apple juice or water. Sucrose and sorbitol solutions (10%) served as positive and negative controls. Plaque pH was measured using a touch microelectrode (Beetrode, NMPH3) at 2 and 5 minutes (min) after eating the cereal. Subjects subsequently consumed 50 ml of whole milk, apple juice or water and their plaque pH values were again measured at 2, 5, 10, 15, 25 and 30 min. For dry FL, sucrose and sorbitol plaque pH was measured at 2, 5 and up to 30 min.

All test foods except sorbitol demonstrated drop in plaque pH. At the end of the test, plaque pH of subjects consuming FL/milk was (6.48 ± 0.30). This value was significantly higher than the values obtained for FL/water (6.02 ± 0.41), FL/juice (5.83 ± 0.49) and FL only (5.83 ± 0.68). The plaque pH of subjects 30 min after rinsing with sucrose or sorbitol solutions

SUMMARY (continued)

were 6.25 ± 0.44 and 6.49 ± 0.37 (repeated measures ANOVA with planned contrasts, $p < 0.005$). Maximum pH drop was observed in test group involving FL/juice followed by test group involving dry FL.

Drinking milk after consuming sugary cereal reduces *in vivo* plaque acidogenicity. If sequenced appropriately between sugary snacks, milk represents a healthy beverage and may benefit oral health.

I. INTRODUCTION

A. Background

Nearly every American has experienced dental caries, the most common oral disease, once in their life time. As reported by the National Health and Nutrition Examination Survey (collected between 1999 and 2004) 92% of adults, between ages 20 to 64 have had caries in their permanent teeth (NIDCR, 2011). According to Centers for Disease Control (CDC), tooth decay affects more than one-fourth of U.S. children the aged 2–5 years and almost half of those between the age of 12 and –15 (CDC, 2011). In addition, there is huge disparity in the distribution of dental caries. The occurrence and severity of dental caries has been reported to be higher among minority, low income and low educated populations (Moynihan and Peterson, 2004; NIDCR, 2011).

Dental caries is a major public health problem and has a profound effect on the general health as well as the quality of life of the affected person. Caries and pain associated with caries can cause problems in eating, chewing, smiling, and socializing. It also leads to restricted activity at school, work, and home resulting in millions of unproductive school and work hours throughout the world (Peterson et al., 2005). The sequelae of oral diseases can cause significant pain and are very expensive to treat. The burden of dental caries lasts a lifetime because once the tooth structure is destroyed; it usually needs restoration and additional maintenance throughout life. Thus the financial cost associated with diagnosis, prevention, treatment and retreatment of oral diseases, particularly dental caries, is very high (Lewis and Ismail, 1995). According to recent CDC data, Americans make about 500 million visits to dentists per year for oral health

related problems and an estimated \$108 billion was spent on dental services in the U.S. in 2010 (CDC, 2011).

B. Oral Health and Underlying Factors

1. Dental caries and plaque

Dental Caries is a chronic microbial process multifactorial in origin. It is a highly preventable disease which can be arrested and potentially reversed in its early stages, but without proper care, it can progress until the tooth is destroyed (Crall, 2006; Selwitz, et al., 2007). The development and progression of dental caries primarily depend on the interactions among diet, oral microflora, and host response (Wu, 2009). Dental caries begins with microbiological shifts within the complex biofilm called dental plaque which covers the tooth surface (Selwitz et al., 2007). Plaque is composed of viable and nonviable bacteria, mucopolysaccharides and other cellular debris and metabolites (Aimutis, 2004). Thus, plaque acts as a biofilm which is a collection of bacterial population held together by a polysaccharide matrix (Marsh, 2004). An appearance of plaque on enamel acts as a first sign of the interaction between tooth and biofilm microorganisms. Dietary composition and host defense mechanism play important roles in maintaining the homeostasis of the plaque biofilm. Saliva and plaque in the oral cavity serves as a host for a variety of microorganisms. Out of several bacterial strains, *Streptococcus mutans* and *Lactobacilli* are important contributors to caries initiation and progression. Increased amounts of fermentable carbohydrates in the diet have shown to increase the level of *Streptococcus mutans* and *Lactobacilli*, in the plaque (Schachtele and Jensen, 1982; Touger- Decker and van Loveren, 2003). However, the role of other oral bacteria present in oral cavity such as non mutans

streptococci (salivarius and sanguis), enterococci, or actinomycetes in caries etiology is not yet clear (Tanzer, et al., 2001).

When food is ingested, plaque bacteria utilize the carbohydrate content in the food to form acids, resulting in a drop in plaque pH and an acidic environment in oral cavity. Acidogenic (acid producing) oral bacteria can result in enamel demineralization. Frequent intake of sugary snacks between meals increases the risk of reducing dental plaque pH for longer intervals leading to more demineralization (Lingstorm, et al., 2000).

2. **Implications of diet**

Diet is one of the important components in modulating oral health. It has been known that the frequency of snacking between meals has an effect on oral health. Most in-between meal snacks are comprised of prepackaged foods which are high in sugar and calories and low in nutrition (Utreja et al., 2008). Sugars are classified into two types. Those which are incorporated in the cellular structure of the food are called intrinsic sugars and those which are added artificially to the food are called extrinsic sugars. Extrinsic sugar plays a crucial role in caries risk. The intake of extrinsic sugar more than four times a day or greater than 60g/person/day for teenagers and adults is considered to increase the rate of caries. Sucrose, an extrinsic sugar also known as table sugar, is considered an important dietary component responsible for the development of caries (Sheiham, 2001). It is highly cariogenic as it is easily fermentable. It serves as a substrate for the synthesis of adherent glucans of the dental plaque matrix which can alter the plaque microflora balance and reduce the plaque pH (Moblely 2003;

Zero, 2004). Sucrose allows synthesis of extracellular and intracellular polysaccharides by oral bacteria in plaque which may serve as a marker for dental caries (Bayrack, et al., 2011).

The higher frequency and consumption of total amount of sugars is important in etiology of caries as it carries a risk of lowering the dental plaque pH for longer intervals. According to U.S. Food Supply Data, per capita consumption of added sugars by Americans increased 23% from 27 tsp./person/day in 1970 to 32 tsp./person/day in 1996 (Johnson and Frary 2001).

A majority of snack products, such as the presweetened, ready to eat (RTE) cereals, have added sugar which increases the total amount of sugar in one's daily diet. Cereals are the most commonly consumed breakfast in the U.S. and other parts of the world. Although traditionally considered breakfast foods, cereals are increasingly being eaten at other times of the day thus adding to its frequency.

Cereals are the combination of refined starch and sugar. According to researchers processed food starches in modern human diets possess a significant cariogenic potential (Lingstorm et al., 2000). Studies have indicated that starch, when consumed with sugars, forms a biofilm which is more cariogenic and porous due to high concentrations of extra-cellular insoluble polysaccharides (IP) (Ribeiro et al., 2005). Thus cereals become a perfect blend of sugar and starch which may accentuate the cariogenic activity of sugars.

Due to the above reasons as well as the wide recognition of cereals as a breakfast food, which constitutes an important daily meal, it is important to assess its oral health potential (Nicklas et al., 1998).

C. **Food Sequencing**

Food sequencing is a relatively newer concept in eating patterns. It is combining of the food items in a meal in an order that facilitates digestion. Food Sequencing is primarily discussed in regards to digestion and weight loss but has been rarely discussed in lieu of oral health. It has been suggested that food sequencing promotes long term health by better managing weight and the digestive system (Rush, 2000). While the order of eating foods is important in aiding digestion, it may affect a person's oral health. For example, it has been reported that cheese reduced plaque acidogenicity after sugar (Rugg-Gunn et al., 1975; Jensen and Wefel, 1990). However, limited information is available regarding the patterns of food combinations on oral health.

D. **Hypothesis**

We hypothesize that the consumption of milk after eating dry sugary cereal will result in a smaller drop in dental plaque pH and promote a less acidic environment in the oral cavity compared to the consumption of fruit juice.

E. **Purpose of the Study**

The purpose of this study is to investigate the effect on plaque acidogenicity in humans after consuming a dry, RTE cereal followed by beverages such as water, fruit juice and whole milk.

II. LITERATURE REVIEW

A. Dental Caries and Sugars

The role of sugars in dental caries is a well-accepted yet controversial topic (Zero, 2004). Sugars can be divided into various groups depending on their structure and chemical properties. It includes monosaccharides such as glucose, fructose, galactose and invert sugar (1:1 glucose and fructose); disaccharides such as sucrose, maltose, lactose (milk sugar) and trehalose; oligosaccharides and polysaccharides such as starch (Moyanihan, 1998). Among all sugars, sucrose has been considered as a special agent in contributing to the dental caries etiology (Newbrun, 1967).

There have been many classical studies which have shown the direct relationship of sugars with dental caries. Stephan was the first to demonstrate the relationship between sugar exposure and low plaque pH in a clinical study. Oral bacteria readily metabolize the sugars and produce organic acids which can result in enamel demineralization (Stephan, 1940). The Vipeholm study confirmed and irrefutably established a direct dose response relationship between sugar and caries. It also pointed out that sugars consumed in between meals have more cariogenic potential than when eaten during meals (Gustafsson et al., 1954). Studies conducted on caries occurrence during and after World War II in Norway and Japan found that the incidence of caries was low during the war when sugar consumption was low due to the shortage in supply; the rate of incidence increased after the war when sugar consumption increased (Toverud, 1957, Takeuchi, 1961).

Another study by Scheinin and colleagues stated that among various sugars, sucrose is more cariogenic than others. It also found out that, when fermentable carbohydrates were replaced by non-fermentable sugar substitutes such as xylitol, caries incidence was reduced (Scheinin et al., 1976). Further, Harris conducted a caries study of Hopewood house in Australia. He found out that the modern diet with added refined sugars was more cariogenic compared to a vegetarian diet comprised of dairy products, fruits and vegetables in a raw or uncooked state and with low amounts of refined sugar diet (Harris, 1963). Thus the modern diet of processed, RTE food and high amounts of refined sugar is of high cariogenic potential. Similar results were found in the Tristan da Cunha study, which examined the influence of change in dietary patterns in people inhabiting a remote island. Caries prevalence increased greatly when a modern diet with sugar and refined carbohydrates was introduced to these residents (Holloway et al., 1963; Fisher, 1968). Newbrun found that the incidence of caries was lower in individuals who suffer from hereditary fructose intolerance, a disease in which individuals must avoid sucrose and fructose (Newbrun et al., 1980; Marthaler, 1967). A report by the Committee on Medical Aspects of Food (COMA) recommended reducing the daily intake of extrinsic sugar and replacing it with starch and fruit to enhance oral health. These and many more studies added to the findings that sugar acts as an important factor in occurrence of caries.

Additional evidence of the positive relationship between sugar consumption and caries was found by national surveys and systematic reviews (Downer, 1999; Zero, 2004). However, recent studies have found that the relationship between sugar and caries is modified in industrialized countries due to the introduction of fluoride in different forms such as tooth paste, oral rinses and common drinking water. A systematic review conducted by Burt and Pai as a part

of a NIH/NIDCR conference on Diagnosis and Management of Dental Caries throughout Life concluded that the relationship between sugar and caries has weakened as compared to during the pre-fluoride era but emphasized that restriction of sugar is still an important and effective measure in caries prevention (Burt and Pai, 2001). Caries remains a serious and growing problem in developing countries that disproportionately affects the economically disadvantaged and recent immigrants in many industrialized nations. There are various factors included in caries etiology, and future research should be directed towards biologic and behavioral factors that influence diet related caries risks (Zero, 2004).

B. Implications of Food and Beverage on Oral Health

Dental caries has been considered a diet dependent microbial infectious disease (Mobley, 2003). The cariogenic potential of any particular food or beverage is influenced by its properties (Bowen et al., 1980). Researchers found that the sugar content of food was not exclusively responsible for enamel demineralization. Starch, flavoring agents and other components of food or snacks also contribute to caries activity (Bibby and Mundroff, 1975).

Starch, a form of carbohydrate, can be found in natural or refined form. Data obtained from studies in animals, humans, and the laboratory; as well as epidemiological data, have supported the low cariogenic potential of starch alone. They confirmed that raw starch has low cariogenic potential but processed or cooked starch exhibits high cariogenic capacity (Grenby, 1967; Bowen et al., 1980; Lingstorm, et al., 1989). Cooked and processed starch can be broken down to release glucose, maltose and maltotriose by salivary amylase. These are trapped on tooth surfaces and act as a source of fermentable carbohydrates to promote acid production by plaque

bacteria (Ribeiro et al., 2005). Kashket and colleagues demonstrated that high starch snack foods were retained longer on tooth surfaces than high sucrose, low starch food (Kashket et al., 1991). Bibby and colleagues evaluated almost 180 different snack foods and beverages for their caries potential and found that foods with higher sugar content did not induce as much enamel demineralization compared to the combination of sugar and starch (Bibby et al., 1986; Pollard, 1995). They reinforced the results of an animal study conducted by Firestone and others which found similar conclusions about starch and sugars in rats (Firestone et al., 1982). The presence of starch in foods with sugars allows increased synthesis of insoluble glucans by glucosyltransferase (GTF) enzyme produced by mutans streptococci, which increases the porosity of the plaque biofilm. The latter facilitates the adherence of cariogenic bacteria such as *Streptococci mutans* and *Actinomyces naeslundii* to the tooth surface (Vacca-Smith et al., 1996).

1. Cereals

As opposed to a primitive diet where starch was consumed alone and used as source of energy, the modern diet is a combination of starch and sugar (Lingstorm et al., 2000). Cereal is an example of such modern and popular breakfast items consumed in the U.S. and other countries around the world. Some varieties of cereals have added sugar which ranges from 0 to 20 g (4 grams of sugar equals 1 teaspoon sugar) thus making it a perfect blend of sugar and starch. Added sugars provide additional calories but carry little nutritional value (NCDHHS, 2011). Some of the reasons to add sugar to prepackaged snack foods are to improve taste, add bulk, and reduce the production cost (Drewnowski, 2003). The cariogenic potential of presweetened cereals and snack foods depends upon their composition, texture, solubility, retentiveness and rate of salivary clearance rather than just sugar content alone (Mobley, 2003).

When sucrose and cooked starch are consumed together the starch bring sucrose closer to the tooth surface for longer period than if it was sucrose alone (Sgan-Cohen et al., 1988) which results in higher pH drop, acid production and ultimately may initiate demineralization. A higher prevalence of caries lesions was noted in children who consumed cereal with sucrose compared to those who did not add sugar to the cereal bowl (Mattos-Graner et al., 1998).

2. **Beverages**

Various studies have been conducted to identify the effect of beverages such as fruit juice and sugar sweetened drinks and milk on oral health. As the eating patterns of the developed nations have changed drastically, more and more people are now inclined to consume processed foods, fruit juices and soft drinks which do not require chewing (Grenby, 1990; USDA, 2001). This has led to diets with more carbohydrates and less fibrous food and in turn more dental decay (Hussein et al., 1996).

a. **Fruit juice**

Fruit drinks are considered healthy food choices, but excessive or improper intake can be a potential risk to dental health as they are not in their natural form and are processed with added ingredients, especially sugar (Kreb-Smith, 2001). The erosive potential of any beverage has been associated with pH, titratable acidity and calcium and fluoride concentrations.

Titratable acidity is the quantity of base required to bring a solution to neutral pH. A higher titratable acidity is relevant with high buffering capacity. Fruit juice such as apple juice has been documented in many studies for its potential for dental erosion due to its highly acidic pH ranging from 2.9–3.6 (Kiran Banan, and Hegde, 2006; Ehlen et al., 2008). The titratable acidity

of apple juice is reported to be approximately 2.9. The pH is considered a stronger predictor of erosion potential than titratable acidity (Murell et al., 2010). The erosive potential of the foods and beverages is increased by the natural occurrence, or addition of acidic compounds such as citric, malic and phosphoric acids (Smith et al., 1997). Fruit juices have been documented for their demineralization and caries progression effect because the acidity produced by apple and orange juice lower the plaque pH comparable to that produced by 10% sucrose solution (Touyz, 1994; Hussein et al., 1996). The critical pH at which enamel dissolves is considered to be below 5.5. Many fruit juices reduce plaque pH well below pH 5.5 are thus having potential for erosion and caries (Touyz, 1994). An in vitro study conducted by Ehlen and colleagues found that 100% apple juice had high potential to erode both coronal and root surfaces of the tooth (Ehlen et al., 2008). A recent study in children reported that children with erosive tooth wear consumed more apple juice, orange/grapefruit juice and soft drinks than others who did not have erosive tooth wear (Okunseri et al., 2011).

b. Milk

In contrast to fruit juices, milk and other dairy products have been reported to be “tooth friendly”. There has been a plethora of research conducted to determine the relationship between dairy products such as milk and oral health. Research from 1950’s sought to understand the beneficial properties of milk in preventing dental caries in humans and animals (McClure, 1953; Merritt et al., 2006). Milk has been reported to be anticariogenic, attributed to its ability to promote tooth remineralization, biofilm inhibition and colonization (Duarte et al., 2000; Oho et al., 2002; Merritt et al., 2006). Whole milk is a good source of calcium, phosphate, proteose-peptone fractions 3 and 5, casein and lipids out of which casein fraction accounts for 80% of

milk protein (Guggenheim et al., 1999; Grenby et al., 2001; Aimutis, 2004). Besides casein, milk contains whey proteins, lactoferrin, antibacterial lysozyme and antibodies which all help to safeguard oral health via their interaction with cariogenic bacteria (Herod, 1994; Duarte et al., 2000). According to the COMA and the findings of many studies, milk and milk sugars (lactose) are non-cariogenic. In an *in vitro* caries model, Bibby et al. reported that adding milk solids to sucrose and starch cakes produced less dissolution of enamel chips by lactic acid-producing bacteria than compared to removing the milk component from the cakes (Bibby et al., 1980). A previous study by Lim demonstrated that routine milk consumption relates to low caries activity (Lim et al., 2008). Another study by Walker also reported the capability of bovine milk to remineralize enamel lesions *in situ* (Walker et al., 2009). Besides human studies, animal studies performed in rats supported the caries protective effect of milk along with the modulatory effect on plaque microbiota (Guggenheim et al., 1999). Non-sweetened dairy products such as cheese, yogurt and milk have been reported to inhibit enamel dissolution and acid production to have beneficial effect on periodontal health (Alzahrani, 2006; Sonmez, and Araz 2007; Shimazaki et al., 2008). However, there remain some concerns regarding lactose content in milk and its role in early childhood caries. Because lactose is a fermentable sugar, conditions such as nursing bottle caries can be caused by the prolonged contact of milk sugar with primary teeth which interferes with the protective actions of saliva (Bowen, 1998). But several studies have repeatedly acknowledged the beneficial properties of milk associated with low caries rates when combined with routine oral hygiene (Petti et al., 1998; Jensen et al., 2000).

C. **Plaque Acidogenicity and Measurement**

There are various ways to assess the acidogenic potential of snacks and foods. The use of the *in vivo* plaque model has been supported by dental scientists and clinicians (Curzon and Hefferen, 2001). Dental plaque is a bio film containing approximately 1×10^{11} bacterial cells per gram of wet weight. It has been estimated that as many as 400 distinct bacterial species may be found in plaque. Oral bacteria present in plaque matrix are very efficient in fermenting carbohydrates in the presence of the anaerobic and localized environment surrounding the tooth surface to produce organic acids and reduce the plaque pH (Schachtele and Jensen, 1982). These acids can lead to demineralization of enamel if produced in enough quantity to reduce the plaque pH below the critical value of 5.5. Thus, measurement of plaque pH response is an important and reliable part of assessment to evaluate the acidogenic as well as the cariogenic potential of various snack foods (Jenkins et al., 1979; Jensen et al., 2000; Eliasson et al., 2006).

The history of dental plaque research can be linked to the 17th century when Antony van Leewenhoek for the first time described the microbial deposits on the teeth (Zaura, and ten Cate, 2004). But the method of measuring the plaque pH to study plaque acidogenicity and assess cariogenic potential was pioneered by Stephan (Stephan, 1940). There are 3 different methods to measure plaque pH, each with specific advantages and disadvantages. Those methods are plaque sampling or harvesting; interproximal telemetry or indwelling electrode; and touch electrode (Preston and Edgar, 2005).

1. **Plaque sampling/harvesting**

Fosdick was the first to develop the method of plaque sampling which was later modified by Englander, Ludwig and Bibby to measure small amounts of plaque scraped from various oral sites (Bibby and Ludwig, 1957). It was further improved by researchers of a Newcastle group involving Edgar, Geddes, Rugg-Gunn and Jenkins. They sampled the plaque from at least 20 intraoral sites and diluted the sampled plaque in distilled water, physiological saline, deionized water or sodium chloride (Rugg-Gunn et al., 1975).

The advantages of the plaque sampling technique include its simplicity, cost effectiveness and flexibility in assessing plaque pH. It can be used in large groups with different ages, health concerns and caries experience (Preston and Edgar, 2005). Although it is used as a standard method for plaque pH measurement, a few issues to administering this method are the accessibility of intra oral sites, the *in vitro* measurement of plaque, the disturbance of the plaque layers, and the process being slow and the difficulty in distinguishing between glycolytic and dietary acids (Schachtele and Jensen, 1982; Curzon and Hefferren, 2001).

2. **Indwelling electrodes/interproximal telemetry**

Graf and Muhelmann devised the technique of interproximal telemetry to overcome the plaque layer disturbance caused by other plaque measurement procedures. They mounted glass and reference electrodes on the removable partial dentures with a power supply. It consisted of miniature transmitters which were used for telemetering the pH of interdental plaque via radio-telemetry (Graf and Muhelmann, 1966). Subsequently, researchers from Zurich

modified the technique to involve insertion of a hollow tooth into a cobalt–chromium mandibular dental prosthesis. A miniature glass electrode was positioned inside the tooth to create a contact point between the hollow denture pontic and the adjacent natural abutment tooth. Subjects were restricted from oral hygiene measures for several days to allow plaque accumulation on the prosthesis. Radio signals were received from the intra-oral device and a continuous plaque pH was recorded (Preston and Edgar, 2005). Imfeld and Reich later described a portable telemetry system for measuring the pH of interdental plaque for several hours in a day without affecting the normal daily activities of the subjects. This was recorded by the miniature pocket recorder (Lingström et al., 1993a). Various groups later modified the original Graf and Muhlemanns telemetric method to suit their individual use.

There are various advantages of this method over others such as the location of electrode at the contact point, continuous monitoring of the plaque pH without disturbing the integrity of plaque layers, and distinction between dietary and glycolytic acids. Utilizing this method, studies can even be performed on aged plaque which is weeks or month old (Schachtele and Jensen, 1982).

Although the indwelling system has the above advantages, it also has a few disadvantages. The method is complex, expensive and technically demanding. The plaque pH is measured at the glass surface and not on the enamel thus it is not natural as bacterial adherence and metabolism factors can be altered (Preston and Edgar, 2005). The electrode is attached to the artificial tooth so at least one missing tooth is required in the oral cavity making it very patient

selective (Schachtele and Jensen, 1982; Lingström et al., 1993a). In addition, the calibration of the indwelling electrode is difficult and not very accurate.

3. **Touch electrodes**

Touch electrodes were first used by Stephan in the 1940's by touching on the labial surfaces of the teeth to monitor the effect on the plaque pH after glucose rinse (Stephan, 1940). These electrodes in 1940's were made of antimony. Stephan found that there was a variation in pH between different individuals and also in different sites in the same individual. He noted that the plaque pH drops from the resting value of approximately 6.5 to around 5 after a glucose rinse and returns back to normal after an interval of approximately 40 minutes (Stephan, 1940). The antimony and carbon electrodes were slow to reach equilibrium, gave fluctuating reading and were prone to error due to oxidizing agents. In 1949, Clement modified Stephan's technique and used a glass electrode with 4.25 mm in diameter instead of antimony. These glass electrodes were impractical to use in the mouth but had high potential for accuracy (Preston and Edgar, 2005). In 1956, Kleinberg reported the use of a modified antimony electrode which used resin as the supporting material for the antimony, to determine interproximal plaque pH (Kleinberg, 1958).

In subsequent years, many modifications and alterations of these touch electrodes were made. In 1980, Liu et al described the palladium- palladium oxide miniature pH electrodes also known as Beetrodes (Liu et al., 1980). Thereafter, various researchers evaluated different types of microelectrodes for intra-oral use. These included the glass microelectrode, the modified bimetallic Beetrode, and the custom-made antimony electrode. The Beetrode, even though

fragile, was considered most suitable for intra-oral use due to its small size, versatility and quick response time. It became the preferred microelectrode for intra-oral use as a touch electrode at the end of the century (Schachtele and Jensen, 1982; Preston and Edgar, 2005). Beetrode has also been reported to exhibit low impedance, which made it rapid in response and suitable for use with portable, battery-powered pH meter thus making it more popular (Harper et al., 1985).

The advantages of touch electrode as mentioned earlier are its simple design and inexpensive set up. It can be used with large numbers of people and can reach interproximal sites easily. It can also monitor pH in certain carious lesions (Schachtele and Jensen, 1982). But touch electrodes share many of the disadvantages of plaque sampling method such as disturbance of the plaque layers and accessibility due to the fragile nature of the electrode. It also measures the pH at plaque saliva interface and cannot differentiate between dietary and glycolytic acids. Sterilization of the electrode has also been considered an important issue in the touch electrodes (Preston and Edgar, 2005).

D. **Food Sequencing:**

Food sequencing or order of eating food is a less explored concept in general. It is combining the food items in a meal in an order that facilitates digestion and helps maintaining body weight. Sequential eating has rarely been discussed in lieu of oral health. One of the medical anthropologist reported that food sequencing promotes long term health by better managing weight and the digestive system (Rush, 2000). While the order of eating foods is important in aiding digestion, it may affect a person's oral health.

Rugg- Gunn et al. (1975) explored the effect of meal patterns on dental plaque pH. They reported that when cheese was eaten after a sugary challenge, the plaque pH responded in low acid production as compared to when eaten before or between meals (Rugg-Gunn et al., 1975). Similar results were reported by Jensen and Wefel, who examined the effects of processed cheese on human plaque pH and de- and remineralization of enamel and root lesions in a human *in situ* caries model system. Their results suggested that cheese consumption prevented the acid challenge when followed by sucrose (Jensen and Wefel, 1990). Another study in 2000 by the same group of researchers found that dairy products such as milk generally reduced the amount of demineralization produced in dentin. Recommendations from this study were to involve milk in combination with other sugary snack to reduce the demineralizing potential of sugary in between meal snacks (Jensen et al., 2000). Although, these studies introduced the concept of food sequencing in dentistry, further investigations are needed.

III. MATERIALS AND METHODS

A. Preliminary Study

Ready to eat dry breakfast cereals are usually consumed together with milk. People consider milk a healthy drink and think that adding milk to a bowl of sugary cereal will help mitigate the negative effects of sugar. However, a preliminary study conducted in Dr. Wu's Laboratory found that the cereal and milk mixture, if let stand at room temperature, had a syrupy consistency. When the plaque pH was measured after consumption of this combination, a reduction comparable to a sucrose challenge was noted. Therefore, in the current study the RTE sugary cereal was consumed dry and not mixed with milk or any other beverage.

B. Study Subjects

A total of twenty healthy adult subjects between the ages of 18-64 were recruited for this study. Subjects were invited to participate with the help of flyers and advertisement posted on the campus of the University of Illinois at Chicago (UIC). Potential subjects were contacted by telephone and the study procedure was briefly explained to them. Once verbally screened for the inclusion and the exclusion criteria, they were asked to come to the College of Dentistry at UIC for the screening visit. Subjects were instructed to refrain from oral hygiene practices for at least 24 hours (hrs) prior to the screening procedure to allow adequate accumulation of dental plaque. They were also asked to refrain from eating anything except water for 2 hrs prior to the testing procedures. At the screening visit, clinical oral examination was conducted to determine the true eligibility of the subject to participate in the study.

1. **Inclusion criteria**

- Be between 18-64 years of age. As cereals are a commonly consumed breakfast food in the U.S, this age group will give a good idea of the effect of various combinations of cereal and beverage on dental plaque pH.
- Give written informed consent to participate in the study and be willing to attend upcoming 6 appointments lasting for 45 min to 1 hr each.
- Be able to refrain from oral hygiene maintenance for 24 hrs prior to the testing procedure. Subjects were also asked to avoid eating/drinking (with the exception of water) for 2 hrs before the test (Pollard, 1995). This criterion was included to avoid the influence of any other dietary components on the plaque pH determination during the test.
- Be in good general health. The subjects should not have any underlying medical condition which may interfere with the testing procedure. For example, diabetes as a diabetic person should not skip breakfast to maintain their sugar level and avoiding eating for 2 hrs before testing may conflict with their schedule.
- Plaque pH should be less than 5.5 after rinsing with a 20% sucrose solution for 1 min. This was an important criterion. It allowed the selection of subjects who responded similarly to sucrose challenge as it varies widely in the general population.

2. **Exclusion criteria**

- Unable to fulfill any inclusion criteria in the above list.
- Absence of natural maxillary premolar teeth on both sides of the jaw.
- Presence of clinically detectable caries or large restorations in the expected testing site. These can again interfere with the plaque pH measurement and may lead to faulty readings.
- Obvious periodontal disease as evidenced by purulent exudate, abscesses, or tooth mobility and or any other signs suggesting that the data collected from that subject may be compromised.
- Having fixed orthodontic appliances in the oral cavity. These are contraindicated as they might interfere with plaque pH measurement by touch electrodes.
- Allergic to any of the cereals/snack foods or beverages being tested, such as lactose intolerance as one of the test food group included consumption of whole milk.

C. **Test Foods**

This study involved 7 visits to the College of Dentistry UIC including an initial screening visit. The test foods included commercially marketed Froot Loops cereal, Dean's Whole milk, Minute Maids 100% apple juice and fountain water. All the commercial products were purchased from the Jewel Osco Departmental Store (Jewel Osco, Chicago IL 60612 USA). From here on, we will refer to the ready to eat, sugar added Froot Loops cereal as 'FL'

The test food groups were:

- Dry FL (20 g)
- Dry FL (20 g) followed by 50 ml of whole milk
- Dry FL (20 g) followed by 50 ml of apple juice
- Dry FL (20 g) followed by 50 ml of fountain water
- 10 ml sucrose solution (10%)
- 10 ml sorbitol solution (10%)

Sucrose and sorbitol solutions were used as positive and negative controls as identified by Human Plaque Acidity Model Working Group (De Paola, 1986). Sorbitol solution is routinely used as a negative control due to its low fermentability and negligible acidogenicity (Takahashi-Abbe et al., 2001).

D. **Study Design**

This study was approved by Institutional Review Board (IRB) at the University of Illinois at Chicago (UIC), protocol number 2009-0420. It was a randomized controlled cross over study. Twenty adult subjects participated in this study. All the qualified subjects were assigned numerical codes. The order of consumption of test food was determined by computer generated randomized sequence. Each subject participated in six study visits including the consumption of four test foods and rinsing with two sugar solutions. Informed consent was obtained from all the participants before the start of the study. Once a subject qualified based on the inclusion and exclusion criteria at the clinic, the baseline plaque pH was recorded. Subjects were instructed to rinse with 20% sucrose solution for 1 min following which the *in vivo* plaque pH was measured

at 2, 5 and 10 min interval. Subjects who demonstrated a pH drop to 5.5 or lower were recruited for the study. The pH 5.5 is considered a critical pH by researchers signifying the threshold value for enamel demineralization. After the initial screening visit, recruited subjects were asked to make six more visits to the college. At those visits, subjects were asked to eat, drink or rinse various test foods following which the plaque pH was monitored and recorded at various time intervals.

Each subject had a minimum of a two day period between two study visits to allow them to return to their routine oral hygiene. At each study visit subjects were instructed to refrain from oral hygiene practice for at least 24 hrs and from eating anything except water 2 hrs prior to the testing procedure similar to the screening visit. Subject's *in vivo* plaque pH was measured at baseline to determine the resting plaque pH. In the study visits involving 10% sucrose or sorbitol solution, subjects were asked to rinse with the solution for one minute and then plaque pH readings were recorded at 2, 5, 10, 15, 20, and 30 min time interval as shown in Figure 1. For study visits involving 20 g FL, subjects were asked to chew and ingest the food within two min and then plaque pH was measured at the above mentioned intervals. For the study visits involving 20 g FL followed by 50ml of whole milk, apple juice or water; subjects were instructed to chew and ingest the dry FL within 2 min. After that the plaque pH readings were recorded at 2 and 5 min as shown in figure 2. Then the subjects were asked to consume 50 ml of milk, juice or water. The plaque pH readings were again recorded at 2, 5, 10, 15, 25, and 30 min time intervals following beverage consumption (Figure 2).

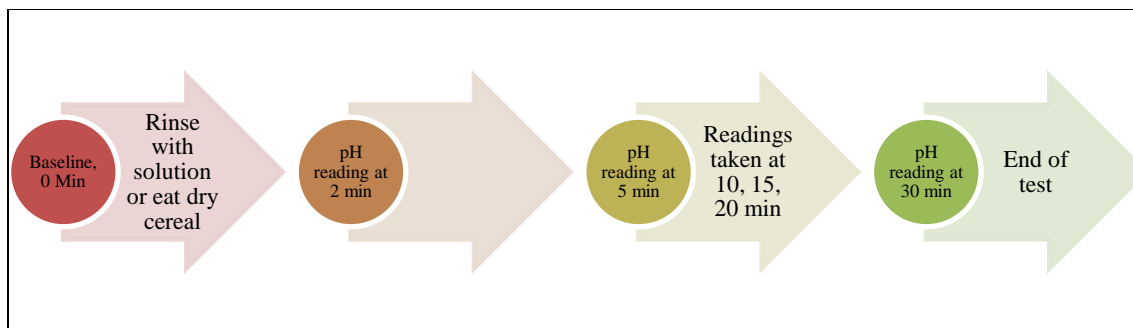


Figure 1. Schematic of time points at which plaque pH reading after rinsing with 10% sucrose, sorbitol solutions and consuming dry FL were taken

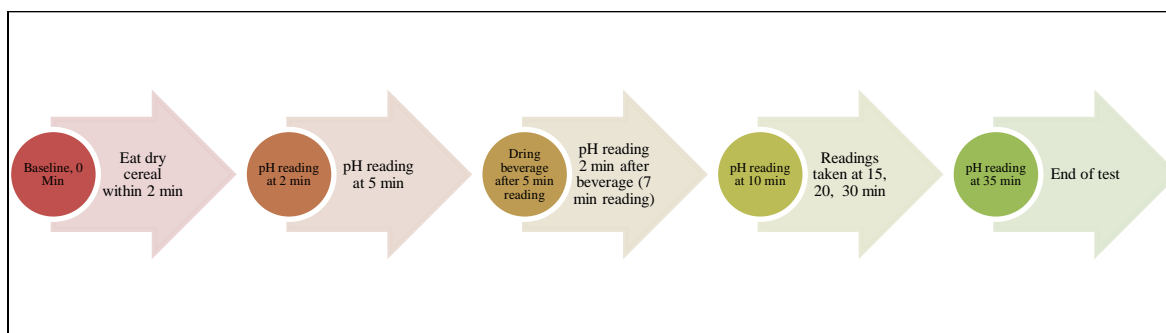


Figure 2. Schematic of time points at which plaque pH reading after consumption of dry Froot Loops cereal followed by whole milk, apple juice or water were taken

E. **Plaque pH Measurement**

The plaque pH of the subjects was measured *in vivo* using a touch microelectrode (Figure 3) (NMPH3, Dental Beetrode®, 45° bend, 2 mm receptacle, marketed by World Precision Instruments [WPI] FL, USA [Figure 4]) and a glass reference electrode (DRIREF -5, WPI). Both electrodes were attached to the pH meter (Figure 5). The microelectrode was calibrated using standard pH buffer solutions of 4.0, and 7.0 before the start of each visit and in between tests. The microelectrode was inserted in to the inter-proximal plaque just below the contact area between the maxillary premolars on both, left and right sides (Figure 6). The reference electrode was kept in a 3M KCL solution. The subject was asked to dip his/her finger in the same 3M KCL solution to create a salt bridge (Figure 7). Once the whole circuit was completed the readings were recorded on the pH meter at various time intervals. The same examiner (S. Naval) measured the plaque pH of every subject at every visit so as to reduce the examiner bias and variability.

F. **Statistical Analysis**

The plaque pH was recorded in the interproximal space of maxillary premolars on both sides in the oral cavity and the mean pH was calculated at each time point for each test group for all subjects. The minimum pH and the difference between resting pH and minimum pH known as maximum pH drop was calculated for each test group with the use of Microsoft Excel. The food groups were compared at each time point using Repeated Measures Analysis of Variance (ANOVA) using SPSS 16.0 for windows, SPSS Inc, Chicago IL, with planned contrasts.



Figure 3. NMPH3 Microelectrode



Figure 4. Dental Beetrode with 45⁰ bend and 2 mm receptacle

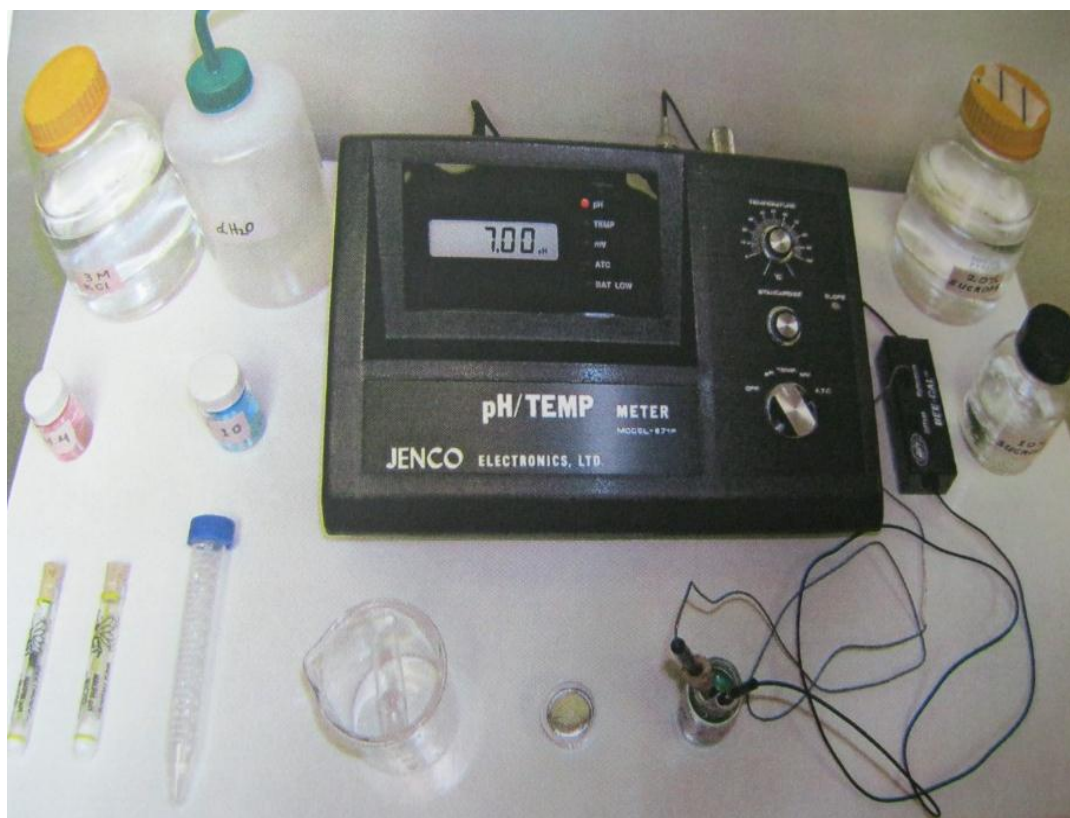


Figure 5. Jenco electronics, JE67IP pH meter



Figure 6. Plaque pH determination in interproximal space of maxillary premolars in subject's oral cavity



Figure 7. Creation of salt bridge in 3M KCL solution

IV. RESULTS

A. Findings of the Study

This randomized controlled cross over study included twenty healthy subjects between 18-64 years of age. (Table I). The mean plaque pH values at baseline and at various time points after consumption of test foods are presented in Table II and Table III. The mean plaque pH data for all test groups represents 20 subjects except dry FL group. Four subjects out of total 20 subjects could not finish the study visit involving dry FL. After repeated contact with them, it was found that subjects had moved out of the city. Therefore, test group involving dry FL represents data for 16 subjects.

The mean baseline or the resting plaque pH (at 0 min) of the subjects ranged from 6.40 ± 0.42 to 6.62 ± 0.40 (Table I, Table II). There was no statistically significant variation noted in the baseline pH values among groups.

The solutions were rinsed for 1 min and dry cereal was chewed and eaten within 2 min. The plaque pH readings were taken at different time intervals as shown in figure 1 and 2. The plaque pH readings over the 30-35 min testing period after rinsing with sugar solutions, eating and/or drinking the test foods and beverages are presented in Table II and III. Except for the 10% sorbitol solution, a drop in plaque pH was noted after consumption of all test foods as measured at 2 min.

TABLE I
SUBJECT DEMOGRAPHICS (n =20)

Race/Ethnicity	Male	Female	Total
Asian	6	8	14
Caucasians	0	4	4
African American	1	1	2

1. **Sorbitol**

Rinsing with sorbitol solution demonstrated the rise in plaque pH from 6.40 ± 0.42 to 6.45 ± 0.36 at 2 min time interval. The lowest pH values were observed between 5 to 15 min after baseline in all of the food groups except sorbitol. Sorbitol did not demonstrate drop in plaque pH over 30 minute time interval. The plaque pH reached to maximum of 6.49 ± 0.37 at the end of 30 min (Table II)

2. **Sucrose**

The sucrose group demonstrated the typical Stephen curve (Figure 8). It showed a drop in the plaque pH from 6.62 ± 0.40 to 6.04 ± 0.49 at the end of 2 min after rinsing. It further decreased up to 5.75 ± 0.48 , 5 min after rinsing and then

followed gradual recovery. At 30 min after baseline, the plaque pH was 6.25 ± 0.44 which remained significantly lower than its baseline pH ($p < 0.001$) (Table II).

3. Dry Froot Loops

There was a steady drop in plaque pH after consumption of dry FL. The lowest pH for dry FL was 5.52 ± 0.45 reached at 10 min after consumption. Although a gradual increase in pH was observed the mean plaque pH value at the end of 30 min was only 5.83 ± 0.68 which was significantly lower than the baseline of 6.56 ± 0.34 ($p < 0.001$) (Table II). This value was also significantly lower than the 30 min reading (6.25 ± 0.44) observed in the sucrose group ($p < 0.024$).

TABLE II

IN VIVO DENTAL PLAQUE PH AFTER RINSING WITH 10% SUCROSE, SORBITOL SOLUTIONS AND CONSUMING DRY FL.

Time (min)	10% Sucrose	10% Sorbitol	Dry FL
0	6.62 ± 0.40	6.40 ± 0.42	6.56 ± 0.34
2**	6.04 ± 0.49	6.45 ± 0.36	6.03 ± 0.48
5	5.75 ± 0.48	6.43 ± 0.42	5.74 ± 0.36
10	5.81 ± 0.51	6.44 ± 0.40	5.52 ± 0.45
15	6.03 ± 0.54	6.45 ± 0.37	5.55 ± 0.59
20	6.16 ± 0.48	6.48 ± 0.40	5.64 ± 0.62
30	6.25 ± 0.44	6.49 ± 0.37	5.83 ± 0.68

*Value represents mean \pm SD

** Two minutes after rinsing with solution or consuming dry FL

4. **Froot Loops/Milk**

In the test group involving dry FL followed by milk, the plaque pH demonstrated an initial drop from baseline value of 6.46 ± 0.34 to 5.75 ± 0.48 at the end of 5 min. This was the lowest pH recorded for the test group (Figure 9). Upon ingesting milk at 5 min after eating FL, the plaque pH gradually increased to 6.48 ± 0.30 at the end of 30 min after drinking milk and was remained close to the initial baseline pH of 6.46 ± 0.34 (Table III). Thus consumption of milk after dry FL did not promote any further drop in plaque pH and helped to bring the plaque pH back to initial baseline pH.

5. **Froot Loops/Water**

In the test group involving dry FL followed by water, upon consuming dry FL, the plaque pH dropped from 6.40 ± 0.34 to 5.75 ± 0.47 at the end of 5 min. It further dropped to 5.57 ± 0.47 , 2 min after consumption of water (7 min reading). After this the plaque pH showed gradual recovery (Table III). At the end of the test (35 min), the pH was 6.02 ± 0.41 and was significantly lower than baseline of 6.40 ± 0.34 ($p < 0.001$).

6. **Froot Loops/Juice**

When subjects drank apple juice after consuming dry FL, the plaque pH was at 5.68 ± 0.42 . It continued the drop to the lowest pH (5.29 ± 0.42) at the end of 10 min (or 5 min after juice ingestion). This plaque pH was also the minimum pH value noted among all test groups and among all testing time points. The plaque

pH gradually rose up to 5.83 ± 0.49 at the end of 35 min, but was still significantly lower than its baseline value ($p < 0.001$) and was also lower than the sucrose group at 30 min (6.25 ± 0.44 , $p < 0.001$). The plaque pH at the end of the test for dry FL and Juice (5.83 ± 0.49) was similar to the pH values of consuming dry FL alone after 30 min (5.83 ± 0.68) (Table II and III). The drinking of juice after dry FL did not promote increase of plaque pH. However, the lowest plaque pH of 5.29 ± 0.42 was lower than the critical demineralizing pH of 5.5 and was also the lowest pH values recorded among all test groups and time points (Figure 10).

TABLE III

IN VIVO DENTAL PLAQUE PH AFTER CONSUMPTION OF DRY FROOT LOOPS CEREAL FOLLOWED BY WHOLE MILK, APPLE JUICE OR WATER

Time (min)	Dry FL/Milk	Dry FL/Water	Dry FL/Juice
0	6.46 ± 0.34	6.40 ± 0.34	6.51 ± 0.30
2**	6.05 ± 0.49	6.04 ± 0.50	6.06 ± 0.41
5***	5.75 ± 0.48	5.75 ± 0.47	5.68 ± 0.42
7	5.85 ± 0.39	5.57 ± 0.47	5.40 ± 0.46
10	5.85 ± 0.35	5.67 ± 0.49	5.29 ± 0.42
15	5.95 ± 0.39	5.72 ± 0.53	5.41 ± 0.48
20	6.12 ± 0.42	5.81 ± 0.49	5.57 ± 0.48
30	6.23 ± 0.38	5.95 ± 0.49	5.65 ± 0.45
35	6.48 ± 0.30	6.02 ± 0.41	5.83 ± 0.49

*Value represents mean \pm SD

** Two minutes after complete consumption of FL.

*** Five minutes after consumption of FL after which, beverage was consumed.

At 5 min after consumption of dry FL, the plaque pH dropped between 5.68 ± 0.42 and 5.75 ± 0.48 in groups involving dry FL and beverage (Table III). The subsequent plaque pH values varied depending on the beverage that was consumed after dry FL. Dry FL + milk was the only test group that showed a recovery of its baseline pH at the end of 30 min similar to the 10% sorbitol group.

The test group involving FL and juice demonstrated minimum pH at 5.15 ± 0.36 . The test group involving dry FL followed by demonstrating minimum pH value at 5.36 ± 0.48 (Table IV). Both of these pH values were lesser than the 10% sucrose group (5.69 ± 0.49).

As seen in Table IV, the maximum value of pH drop was observed in FL and Juice test group (1.36 ± 0.30). Maximum pH drop was measured as a difference between the resting plaque pH and the minimum pH during the test. Subjects who consumed dry FL showed the second highest value of maximum pH drop (1.19 ± 0.33) similar to the dry FL and Juice group. The maximum pH drop in sucrose group was 0.93 ± 0.37 which was more than the remaining 3 test groups.

TABLE IV

MEAN OF MINIMUM DENTAL PLAQUE PH AND MAXIMUM PH DROP IN DIFFERENT TEST GROUPS

Test food	Baseline pH	Minimum pH	Maximum pH drop
10% Sucrose	6.62 ± 0.40	5.69 ± 0.49	0.93 ± 0.37
10% Sorbitol	6.40 ± 0.42	6.31 ± 0.39	0.09 ± 0.08
Dry FL	6.56 ± 0.34	5.36 ± 0.48	1.19 ± 0.33
Dry FL/Milk	6.46 ± 0.34	5.66 ± 0.38	0.80 ± 0.35
Dry FL/Water	6.40 ± 0.34	5.48 ± 0.46	0.92 ± 0.38
Dry FL/Juice	6.51 ± 0.30	5.15 ± 0.36	1.36 ± 0.30

*Value represents mean ± SD

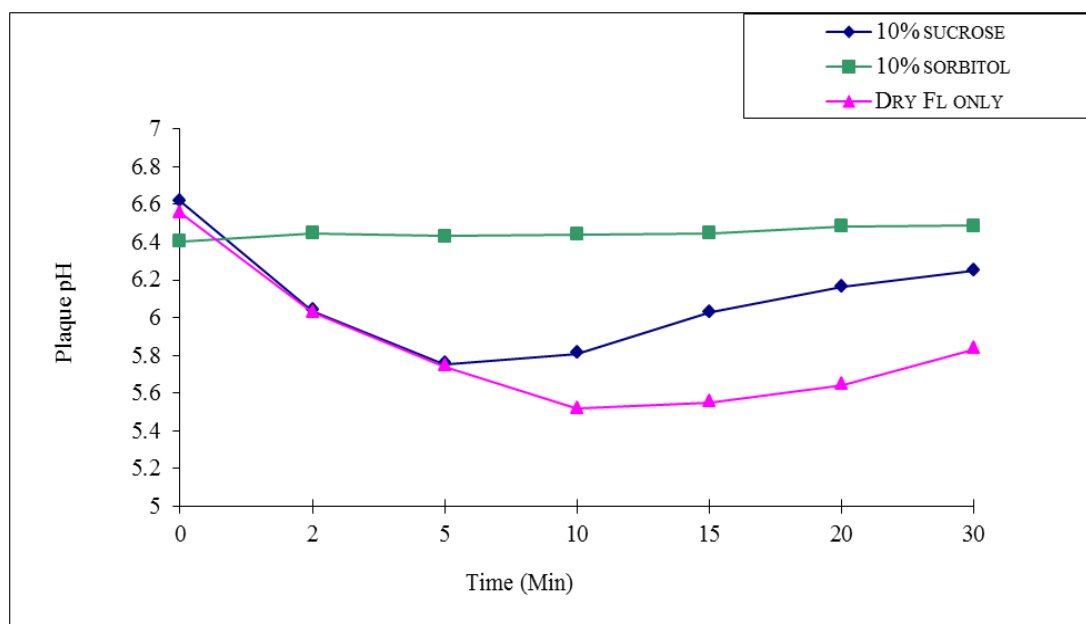


Figure 8. *In vivo* dental plaque pH changes after rinsing with 10% sucrose, sorbitol solutions and consuming dry Froot Loops

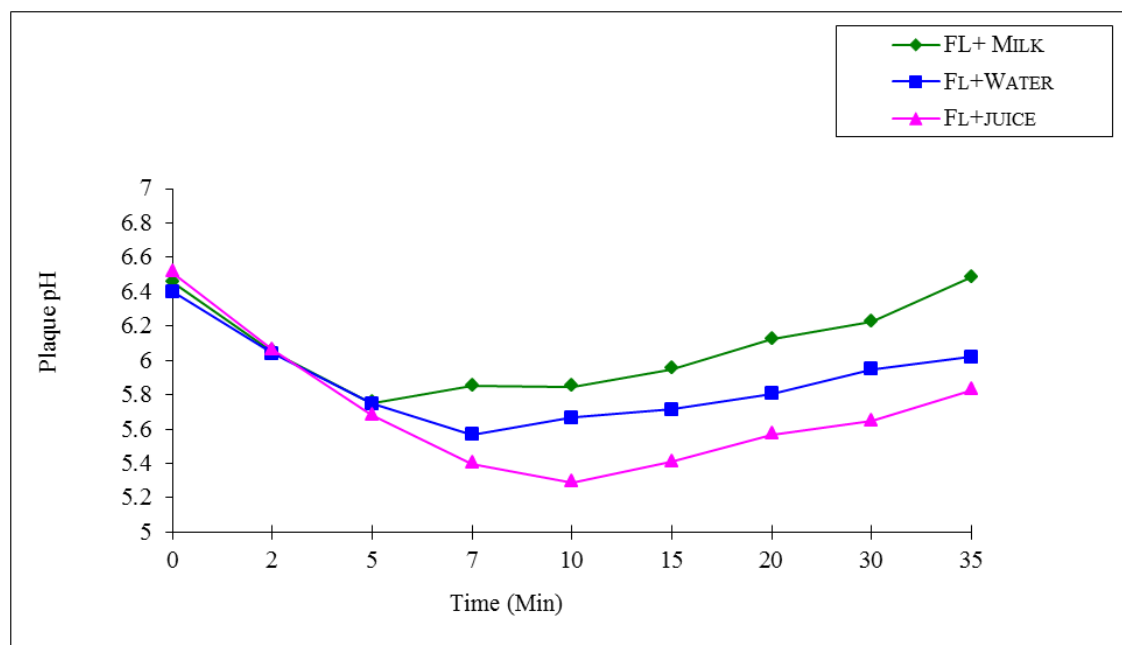


Figure 9. *In vivo* dental plaque pH changes after consumption of dry Froot Loops cereal followed by whole milk, apple juice or water

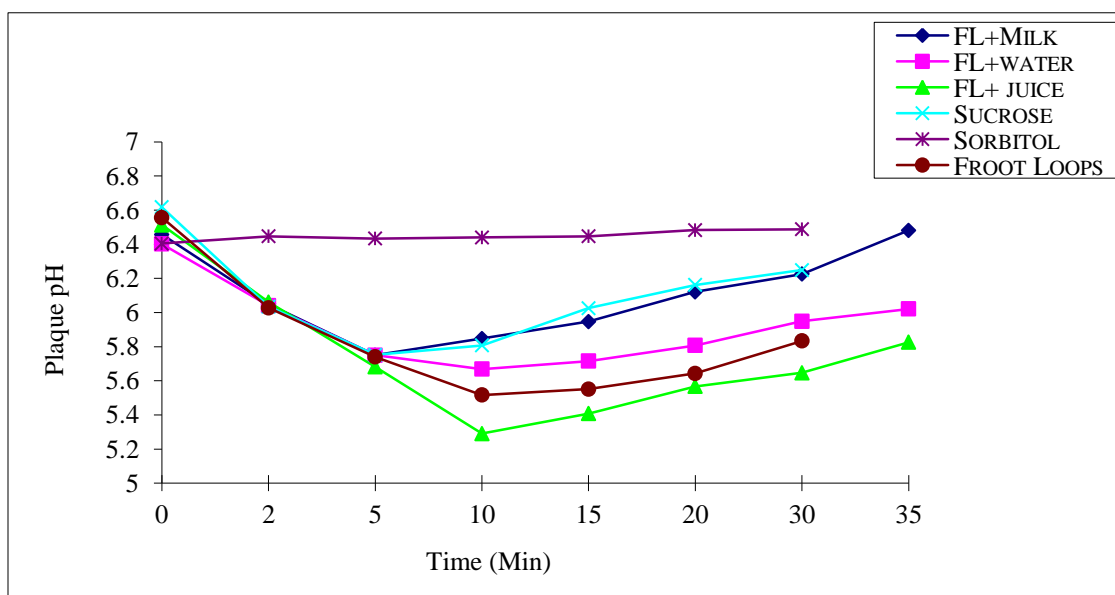


Figure 10. *In vivo* dental plaque pH after consumption of test foods

V. DISCUSSION

Dental plaque is a diverse community of micro-organisms found on the tooth surface as a biofilm, embedded in an extra-cellular matrix of polymers of host and microbial origin (Marsh, 2004). There are three theories proposed to establish the relationship between plaque bacteria and caries. The "Specific Plaque Hypothesis" states that out of the diverse collection of bacteria in plaque only few bacterial species are actively involved in disease. In contrast, the second theory, "Non-Specific Plaque Hypothesis", considers that disease is the outcome of the overall activity of the total plaque microflora resulting from interaction between various bacterial species. The third and most recent "Ecological Plaque Hypothesis" suggests combination of the earlier two theories. It states that cariogenic bacteria are naturally present in healthy oral cavities, but not at clinically relevant levels, and that disease results from shifts in the balance of the resident plaque microflora. For example, frequent intake of sugary food results in the lowering of plaque pH, which may select for bacteria such as mutans streptococci or lactobacilli. These bacteria may produce even more acid, leading to enamel demineralization (Bowen, 1976; Marsh, 2006).

The strong linkage between the caries, gingivitis and acidic environment created by plaque bacteria testifies for measurement of dental plaque pH to aid in understanding the acidogenic potential of foods and beverages (Edgar, 1982; Marsh, 1994; Rugg-Gunn et al., 1975). There are three established methods to measure dental plaque pH: touch electrode, plaque sampling, and indwelling electrode telemetry (Curzon and Hefferren, 2001; Lingstrom et al., 1993b). Beetrodes, made up of iridium-iridium are much more refined than the antimony touch

electrode introduced initially by Stephen (Preston and Edgar, 2005). In this study, we used the touch electrode method using Beetrode as it is convenient, portable, time saving, and can measure *in vivo* plaque pH changes. The tip of this microelectrode is 0.1 mm in diameter which eases placement in the interproximal areas of tooth to monitor plaque pH (Arasb, 2007). However, the delicate and fragile nature of the microelectrode demands a steady grip by the examiner to avoid fluctuating readings and breakage of the electrode tip. In this study, the plaque pH was recorded at frequent intervals during the Stephen curve response to capture adequate data including minimum pH which is often noted in first 10 min (Curzon and Hefferren, 2001).

It is well known that diet plays an important role in the predisposition of dental caries. Earlier literature has emphasized that excess as well as frequent sugar consumption is an important etiological factor for caries occurrence (Curzon and Hefferren, 2001; Sheiham, 2007). Through human and animal studies, researchers have stressed that cariogenicity of food is dependent upon its composition, retentiveness, solubility and rate of salivary clearance (Jensen et al., 2000; Mobley, 2003). A positive association between intake of modern refined carbohydrates and high levels of microbial activity has been well documented since the Vipeholm study (Edgar, 1982; Marsh, 1994; Gustafsson et al., 1954). Researchers found that starch, flavoring agents and other components of food also contribute to caries activity (Bibby and Mundroff, 1975). The effect of starch on dental plaque pH has been discussed for many years. Starches have low acidogenic potential as compared to sugar when used as a sole source of carbohydrate in the diet (Lingstorm et al., 1993a). However, processed starch can be rapidly broken down to release glucose, maltose and maltotriose by salivary amylase in the oral cavity which can act as a source of fermentable carbohydrates to promote acid production resulting in low plaque pH. Also,

when starch is consumed with sugar, it forms porous and cariogenic biofilm due to high concentration of extra cellular insoluble polysaccharides (IP) which may change the composition of plaque matrix making it more caries promoting (Kashket et al., 1994; Ribeiro et al., 2005; Bayrak et al., 2011).

Cereals are the popular breakfast food usually consumed with milk. If eaten dry then it is generally followed by any beverage such as milk, juice, or water. The results of our preliminary study found that cereal when combined with milk, behaved comparable to 10% sucrose solution. Therefore, we decided to use dry cereal followed by most commonly consumed beverages namely milk, apple juice and water.

It was noted that when the consumption of sweetened dry cereal was followed by beverage such as milk or juice, the acidogenicity of plaque was altered depending on the type of beverage consumed. Throughout the study, plaque pH values recorded at the baseline for subjects of each test group did not vary significantly indicating their reproducibility as reported by previous studies (Tahmassebi and Duggal, 1996).

Sucrose and sorbitol solutions were used as positive and negative controls respectively. All the test groups, except sorbitol, exhibited drop in plaque pH after their consumption. A marked decrease in the plaque pH was observed in dry FL cereal group. The FL cereal chosen in this study contains 12 g of sugar as per the package label. It represents the typical combination of sugar and starch. Many studies have reported that consumption of cereal results in decrease in interproximal plaque pH (Mundorff et al., 1990; Park et al., 1990; Pollard et al., 1996; Utreja et

al., 2009) which is supported by our current findings. Ten min after consumption of dry FL, the plaque pH was reduced almost to the critical pH value for demineralization. It was noted that the plaque pH stayed more acidic than the 10% sucrose test group after 30 min. This suggests that dry cereal is more acidogenic compared to sucrose solution under our testing conditions (Table II). Further investigations are warranted to evaluate the acidogenic potential of other ready to eat sugary cereals.

Consumption of FL followed by apple juice, demonstrated maximum drop in plaque pH below demineralization levels at 10 min. This high drop can be attributed to the double challenge of sugars as FL and apple juice both contains added sugars (Pollard, 1995; Pollard et al., 1996). This result was consistent to past studies. This was expected with apple juice due to its known properties of being equally acidogenic as 10% sucrose solution (Hussein et al., 1996; Saeed and Al-Tinawi, 2010), acidic pH and high potential for dental erosion, and decay (Ehlen et al., 2008; Touyz, 1994).

In this study we chose a commonly consumed apple juice and evaluated its acidogenic potential after consumption of dry cereal. Further research with other juices such as orange and grape should be done to evaluate their acidogenic potential in food combination studies.

Water represents the most common drink after any food. Drinking water after dry FL resulted in the minimum plaque pH at 7 min which was intermediate between the milk and juice groups (Table III). This might have been due to the fact that plain fountain water contains no sugar and has none of the beneficial properties of milk. However, drinking water is helpful in

clearing sugars from the oral cavity, thus reducing the amount of acid produced by oral bacteria. Although water did not restore the plaque pH to its baseline, the minimum pH was above the critical pH of 5.5.

Cereal is generally consumed with milk. As per our preliminary data where we found the plaque pH response after adding milk to cereal was similar to 10% sucrose we wanted to find out its effect when consumed after the sugary cereal. Initially after consumption of dry cereal, the plaque pH dropped in the first 5 min but when milk was ingested at 5 min, the plaque pH did not drop further (Table III). This marked an important finding in this study. It may be explained by the many beneficial properties of milk listed previously including: its buffering capacity, protein adsorption on tooth surface and its ability to expedite the clearance of sugars from the oral environment (Levine, 2001, Merritt et al., 2006). Milk and dairy foods such as cheese has been reported to have anticariogenic action. They have high calcium and phosphate content which benefit oral health (Rugg- Gunn et al., 1975; Jensen et al., 2000). Thus milk when consumed after a high sugary cereal challenge did not allow further pH drop and demonstrated a protective role towards teeth by raising the plaque pH even higher than the baseline.

The findings from maximum pH drop analysis also support the same results as above. The highest value of maximum pH drop was observed in dry FL and juice group followed by dry FL then 10% sucrose. The dry FL and milk group showed the lowest pH drop except for the 10% sorbitol.

The acidogenicity and demineralizing potential of individual foods such as dairy products, fruit juices, snack foods and sugars have been previously reported (Pollard, 1996; Jensen et al., 2000). But there is limited literature on the effect of combinations of foods that occur in typical breakfasts or other meals on dental plaque pH. For example, it is not clear whether the cariogenicity of sugars can be modified when it is followed by non-cariogenic food. The usual dietary advice for preventing dental decay is to limit frequent intake of sugars. Very seldom emphasis has been given as to how the cariogenic potential of sugars can be mitigated by other foods. Our findings show that plaque pH was lowered well below the critical levels of 5.5 after the consumption of FL followed by juice but was returned to above baseline pH level when milk was consumed after FL.

VI. CONCLUSIONS

- The effect of test cereal and beverages on plaque acidogenicity was:
- FL/Juice > FL > 10% Sucrose > FL/Water > FL/Milk > 10% Sorbitol
- Consumption of RTE sugary cereal (FL) resulted in a reduction in dental plaque pH which was significantly greater than that obtained after rinsing with 10% sucrose solution.
- Consumption of juice after eating FL resulted in lowering plaque pH to below the critical pH and did not regain the baseline pH at the end of testing period.
- Consumption of milk after FL did not promote drop in plaque pH and raised it above baseline pH at the end of the test.
- Consumption of water after FL did not promote excessive drop in pH and brought the pH back to just above 6 at the end of the test.
- Drinking milk after consuming sugary cereal reduces *in vivo* plaque acidogenicity.
- Milk represents a healthy beverage and may benefit oral health if sequenced appropriately between sugary snacks.
- There is a need to develop educational material and design programs to raise awareness regarding the consumption of sugary cereals/ snacks, food sequencing and maintaining good oral health.

The results from this study give important information regarding acidogenicity of dry sugary cereals in the oral cavity when followed by different beverages and adds knowledge

regarding sequencing of food. The findings of this study may be used as an estimate in future studies to determine potential carcinogenicity of other combinations of foods.

Further research in the understanding of the effects of combinations of foods or food sequencing on plaque acidogenicity is warranted.

CITED LITERATURE

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APPENDICES

APPENDIX A

UNIVERSITY OF ILLINOIS AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

Approval Notice Continuing Review

May 16, 2011

Christine D. Wu, MS, PhD
Pediatric Dentistry
801 S. Paulina St., 469J Dent. Bldg.
M/C 850
Chicago, IL 60612
Phone: (312) 996-7531 / Fax: (312) 996-1981

RE: **Protocol # 2009-0420**
“Plaque Acidogenicity Resulting from Beverages Consumed after Sugary Cereal”

Dear Dr. Wu:

Your Continuing Review was reviewed and approved by the Expedited review process on May 5, 2011. You may now continue your research.

Please note the following information about your approved research protocol:

Please note that training credits for Principal Investigator, Christine Wu will expire on June 15, 2011. All UIC investigators and key research personnel involved in human subject research must complete a minimum of two hours of continuing education in human subject protection every two years. For further information, please see the OPRS

<u>Protocol Approval Period:</u>	May 5, 2011 - May 3, 2012
<u>Approved Subject Enrollment #:</u>	30 (20 subjects enrolled-limited to data analysis)
<u>Additional Determinations for Research Involving Minors:</u>	These determinations have not been made for this study since it has not been approved for enrollment of minors.
<u>Performance Sites:</u>	UIC
<u>Sponsor:</u>	Department
<u>PAF#:</u>	Not available
<u>Grant/Contract No:</u>	Not available

APPENDIX A (continued)

Grant/Contract Title: Not available

Research Protocol(s):

- a) Plaque acidogenicity resulting from beverages consumed after sugary cereal; Version 3, 12/8/09

Recruitment Material(s):

N/A-limited to data analysis

Informed Consent(s):

N/A-limited to data analysis

Your research meets the criteria for expedited review as defined in 45 CFR 46.110(b)(1) under the following specific category:

(4) Collection of data through noninvasive procedures (not involving general anesthesia or sedation) routinely employed in clinical practice, excluding procedures involving X-rays or microwaves. Where medical devices are employed, they must be cleared/approved for marketing. (Studies intended to evaluate the safety and effectiveness of the medical device are not generally eligible for expedited review, including studies of cleared medical devices for new indications.)

Examples: (a) physical sensors that are applied either to the surface of the body or at a distance and do not involve input of significant amounts of energy into the subject or an invasion of the subject's privacy; (b) weighing or testing sensory acuity; (c) magnetic resonance imaging; (d) electrocardiography, electroencephalography, thermography, detection of naturally occurring radioactivity, electroretinography, ultrasound, diagnostic infrared imaging, doppler blood flow, and echocardiography; (e) moderate exercise, muscular strength testing, body composition assessment, and flexibility testing where appropriate given the age, weight, and health of the individual.

Please note the Review History of this submission:

Receipt Date	Submission Type	Review Process	Review Date	Review Action
05/02/2011	Continuing Review	Expedited	05/05/2011	Approved

Please remember to:

→ Use your **research protocol number** (2009-0420) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with all requirements on the enclosure,
"UIC Investigator Responsibilities, Protection of Human Research Subjects"

Please note that the UIC IRB has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your

APPENDIX A (continued)

research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact OPRS at (312) 996-1711 or me at (312) 996-0548. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Brandi L. Drumgole, B.S.
IRB Coordinator, IRB # 1
Office for the Protection of Research

Subjects

Enclosure(s):

- 1. UIC Investigator Responsibilities, Protection of Human Research Subjects**
- 2. Data Security Enclosure**

cc: Indru C. Punwani, Pediatric Dentistry, M/C 850
OVCR Administration, M/C 672

APPENDIX B

University of Illinois at Chicago
Consent for Participation in Research
“Plaque acidogenicity resulting from beverages consumed after sugary cereal”

Why am I being asked?

You are being asked to be a subject in a research study about the effect of consuming selected cereals, milk and fruit juice on dental plaque. This study is conducted by Dr. Christine D. Wu at the University of Illinois at Chicago (UIC) College of Dentistry. We ask that you read this form and ask any questions you may have before agreeing to be in the research.

Your participation in this research is voluntary. Your decision whether or not to participate will not affect your current or future relations with the University or the College of Dentistry. If you decide to participate, you are free to withdraw at any time without affecting that relationship.

Why is this research being done?

Tooth decay or dental caries is a very serious problem that results from an increased production of acid by bacteria in the mouth. This acid destroys the tooth structure and produces cavities. Different bacterial species are present on the surfaces of teeth in the form of dental plaque. Some of these species break down sugars and other food components to produce acid when foods rich in carbohydrates are consumed. As acid production by bacteria in dental plaque is an important factor leading to the development of dental caries, we wish to measure dental plaque pH after consumption of commercially marketed cereal such as Froot Loops, juice and milk.

Dental plaque is a soft deposit that accumulates on the teeth. The pH scale measures acidic or basic nature of substance. It ranges from 0- 14. A pH of 7 is neutral, a pH less than 7 is acidic and a pH greater than 7 is basic.

What is the purpose of this research?

The purpose of this research is to measure the acid produced by bacteria in dental plaque after eating selected cereals, milk and fruit juice.

What procedures are involved?

If you agree to be in this research, we would ask you to do the following things:

Approximately 30 subjects will be involved in this research at the UIC.

1. During the initial screening, the initial dental plaque pH will be measured by placing an electrode which is a small device use to measure dental plaque pH in the area between the upper premolar teeth on each side of your mouth.
2. You will then be asked to rinse with 10 milliliter (which is approximately two teaspoons) sugar solution (20%) for 1 minute, following which dental plaque pH will be measured again at 2, 5 and 10 min. A drop in plaque pH value to ≤ 5.5 will qualify you for the research.

APPENDIX B (continued)

3. Before every testing visit, you will be asked not to brush/floss 24 hours prior to the test and not eat/drink 2 hours prior except for plain water.
4. You will have six study visits. In every visit one test food will be tested. The test food groups will be 10% sucrose solution, 10% sorbitol solution, dry Froot Loops cereal followed by whole milk, dry Froot Loops followed by water, dry Froot Loops followed by apple juice and dry Froot Loops only. Sucrose and sorbitol solutions are routine consumable purified water sugar solutions that are found in everyday snacks and foods.
5. The quantity of 10% sucrose and sorbitol solutions will be 10 milliliter (approximately 2 teaspoons), for dry Froot Loops cereal will be 20 grams (approximately half cup) and for milk apple juice and water will be 50 milliliter (approximately half cup)
6. On the day of testing, initial dental plaque pH will be measured by placing the electrode in the same areas as the previous visit.
 - a. For the test food groups which involve rinsing with 10% sucrose, 10% sorbitol solution or eating dry Froot Loops cereal, your plaque pH reading will be recorded at 2, 5, 10, 15, 20, and 30 minutes.
 - b. For the test food groups which involve Froot Loops followed by whole milk, apple juice or water, plaque pH reading will be recorded after eating Froot Loops. Then you will be asked to drink either, whole milk, apple juice or water depending on the assigned group. After drinking the one of the beverages, your plaque pH will be measured again at 2, 5, 10, 15, 20 and 30 minutes.
7. This study involves total of 7 visits including the screening visit, to the College of Dentistry.

What are the potential risks and discomforts?

There is no known risk from dental plaque pH tests. During plaque pH measurement, the placement of the electrode might cause slight discomfort.

Are there benefits to taking part in the research?

There are no direct benefits to you. The knowledge gained from this study could benefit the society at large by helping in the categorization of foods and beverages that are harmful to teeth.

Will I be told about new information that may affect my decision to participate?

During the course of the study, you will be informed of any significant new findings (either good or bad), such as changes in the risks or benefits resulting from participation in the research or new alternatives to participation, that might cause you to change your mind about continuing in the study. If new information is provided to you, your consent to continue participating in this study will be re-obtained.

APPENDIX B (continued)

What about privacy and confidentiality?

The only people who will know that you are a research subject are members of the research team. No information about you, or provided by you during the research will be disclosed to others without your written permission, except:

- If necessary to protect your rights or welfare (for example, if you are injured and need emergency care or when the UIC Institutional Review Board monitors the research or consent process); or
- If required by law.

When the results of the research are published or discussed in conferences, no information will be included that would reveal your identity.

What are the costs for participating in this research?

There are no costs for participating in this research.

Will I be reimbursed for any of my expenses or paid for my participation in this research?

You can get up to maximum 130 dollars if you complete the study. Each visit will be approximately 45 minutes long. You will be paid \$10 for screening visit. If qualified for the study, you will be paid \$20 in cash after the completion of each testing visit.

Can I withdraw or be removed from the study?

Your participation in this research is VOLUNTARY. If you choose not to participate, that will not affect your relationship with UIC or your right to health care or other services to which you are otherwise entitled. If you decide to participate, you are free to withdraw your consent and discontinue participation at any time without affecting your future care at UIC.

The investigator may withdraw you from participating in this research without your consent if circumstances arise which warrant doing so. If you become ill during the research, you may have to drop out, even if you would like to continue. The investigator, Dr. Wu, will make the decision and let you know if it is not possible for you to continue.

Who should I contact if I have questions?

The researcher conducting this study is Dr. Christine D. Wu. You may ask any questions you have now. If you have questions later, you may contact the researcher at: (312) 355-1610.

What are my rights as a research subject?

If you feel you have not been treated according to the descriptions in this form, or you have any questions about your rights as a research subject, you may call the Office for the Protection of

APPENDIX B (continued)

Research Subjects (OPRS) at 312-996-1711 (local) or 1-866-789-6215 (toll-free) or e-mail OPRS at uicirb@uic.edu.

What if I am a UIC student?

You may choose not to participate or to stop your participation in this research at any time. This will not affect your class standing or grades at UIC. The investigator may also end your participation in the research. If this happens, your class standing or grades will not be affected. You will not be offered or receive any special consideration if you participate in this research.

What if I am a UIC employee?

Your participation in this research is in no way a part of your university duties, and your refusal to participate will not in any way affect your employment with the university, or the benefits, privileges, or opportunities associated with your employment at UIC. You will not be offered or receive any special consideration if you participate in this research.

Remember:

Your participation in this research is voluntary. Your decision whether or not to participate will not affect your current or future relations with the University. If you decide to participate, you are free to withdraw at any time without affecting that relationship.

You will be given a copy of this form for your information and to keep for your records.

Signature of Subject

I have read the above information. I have been given an opportunity to ask questions and my questions have been answered to my satisfaction. I agree to participate in this research. I have been given a copy of this form.

Signature

Date

Printed Name

Signature of Researcher

Date (must be same as subject's)

Printed name of Researcher

APPENDIX D

Advertisement for the study

Research Participants Needed

Study Title:

“Plaque acidogenicity resulting from beverages consumed after sugary cereal”

Who: Males and females between the ages of 18-64 in good health.

UIC College of Dentistry is conducting a research study on the effect of cereal and beverages on dental plaque.

All qualified participants will be compensated for their participation in this research study. The total of seven visits including the screening visit will be required to complete the study. Each testing visit will be for approximately 45 minutes in length.

Interested?

Please contact Shilpa Naval (Study Coordinator) at **312-413-8583** or email at snaval2@uic.edu

Principal Investigator:
Dr. Christine D. Wu
Department of Pediatric Dentistry
College of Dentistry
University of Illinois at Chicago
IRB protocol 2009-0420

VITA

NAME: Shilpa Kshitij Naval

EDUCATION: M.S., Oral Sciences, University of Illinois at Chicago, 2011

M.P.H., School of Public Health, University of Illinois at Chicago, 2011

B.D.S., Government Dental College and Hospital, University of Nagpur, India, 2002

WORK EXPERIENCE: Graduate Assistant, Department of Psychiatry at the University of Illinois at Chicago, Chicago, IL, 2008-2011

Graduate Research Assistant, Department of General Internal Medicine at the Northwestern University and Hospital, Chicago, IL, 2011

Graduate Research Assistant, College of Dentistry at the University of Illinois at Chicago, Chicago, IL, 2008-2010

Associate Dentist, 'Siddhesh' Clinic, Jalgaon, India, 2004-2005

Associate Dentist, Godavari Dental Clinic, Chandrapur, India, 2003-2004

Clinical House Officer, Government Dental College and Hospital, University of Nagpur, India, 2002-2003

Intern, Government Dental College and Hospital, University of Nagpur, India, 2001-2002

HONORS: Inducted as a student member in Delta Omega Honorary Society of Public Health for excellent academic achievement and community leadership in MPH curriculum

UIC Research Forum Life Sciences Research Award- University of Illinois at Chicago, Chicago, IL, 2010

Young Investigator Clinical Sciences Award- American Association for Dental Research, Chicago Chapter, 2010

Gold Medal for highest aggregate in four year Bachelor of Dentistry curriculum, University of Nagpur, India, 2002

Best Student Academic Award - University of Nagpur, India 2002

Gold Medal for highest aggregate in fourth year B.D.S., University of Nagpur, India, 2001

Periodontics Award- Indian Society of Periodontics, India, 2001

Silver Medal for highest aggregate in third year B.D.S., University of Nagpur, India, 2000

Gold Medal for highest aggregate in second year B.D.S., University of Nagpur, India, 1999

Colgate-Palmolive Scholarship- Second Year B.D.S., India, 1999

Gold Medal for highest aggregate in first year B.D.S., University of Nagpur, India, 1998

**PROFESSIONAL
MEMBERSHIPS:**

American Academy of Pediatric Dentistry

American Association of Dental Research

American Public Health Association

American Association of Public Health Dentistry

Indian Dental Association

**ABSTRACTS/
PRESENTATIONS:**

Naval, S., Dave, S., Kandula, N. Community Based Participatory Research: A Study to Understand the Physical Activity Behaviors in Chicago's South Asian Community. Research Day, School of Public Health, University of Illinois at Chicago, Chicago, IL, 2011

Naval, S., and Wu, C. Effect of Sugary Snacks and Food Sequencing on Oral Health, School of Public Health, University of Illinois at Chicago, Chicago, IL, 2010

Naval, S., Salzman, L., Koerber, A., Punwani, I., Johnson, B., Wu, C. Plaque Acidogenicity Resulting from Beverages Consumed after Dry Sugary Cereal. Clinic and Research Day, University of Illinois at Chicago, Chicago, IL, 2010

Naval, S. Medical Emergencies in Dental Field. Annual Maharashtra State Dental Conference, Aurangabad, India 2000.