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JULY 2023

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Arginine and the Healthy Oral Microbiome

Proceedings from a Colgate Symposium: Arginine–A Breakthrough Technology Fighting the Caries Epidemic

Sponsored Outsert

This JADA+ Monograph presents summaries of presentations from a Colgate Symposium: Arginine–A Breakthrough Technology Fighting the Caries Epidemic at IADR in July 2021. The articles did not undergo peer review. Publication of this outsert was supported by Colgate-Palmolive Company.

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ARGININE AND THE HEALTHY ORAL MICROBIOME

Proceedings from a Colgate Symposium:
Arginine - A Breakthrough Technology Fighting the Caries Epidemic. 07/22/2021

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ARGININE: A BREAKTHROUGH TECHNOLOGY FIGHTING THE CARIES EPIDEMIC

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Introduction

I am delighted to write the editorial introduction to this special outsert of *JADA* composed of a series of papers that explores and describes the use of arginine as a new active in the fight against dental caries. Each of these papers is based on presentations by the authors at a Colgate Symposium at IADR titled Arginine: A Breakthrough Technology Fighting the Caries Epidemic on July 22, 2021.

This summary provides the briefest overview of the manuscripts presented in this collection, which I would encourage you to read in detail. The collected scientific knowledge and expertise that the authors bring to this special monograph make the collection an important resource for students, faculty, and experienced practitioners alike.

Caries: Defining the Disease and Its Continued Impact—Pitts and Wolff

Nigel Pitts, BDS, PhD, FRSE opens this special edition with a detailed examination of the current theories surrounding the etiology and natural history of dental caries[1]. He begins with a succinct definition of the disease, which, as quoted, provides the conceptual framework for each of the papers included in this collection:

> Dental caries is a biofilm-mediated, diet modulated, multifactorial, non-communicable, dynamic disease resulting in net mineral loss of dental hard tissues [Fejerskov 1997; Pitts et al., 2017]. It is determined by biological, behavioural, psychosocial, and environmental factors. [Because of this] process, a caries lesion develops.

This quote defines caries as a *dynamic process*—one in which an almost continual process of demineralization and remineralization is occurring within our patients' mouths—the balance of which can be influenced by a range of factors, each of which is described in this elegant definition. Pitts goes on to link this definition to the management of this balance described as "caries care, management and control," in which actions are taken to favor the remineralization side of the caries process, be they by professional, chemical, patient, or environmental interventions. Both Dr. Nigel Pitts et al.[1] and Dr. Mark S. Wolff et al.[2], in the second paper in the monograph, remind us of the ongoing importance of dental disease and its global impact.

We are reminded that caries is a lifelong disease—not one restricted to children—where risk extends into adulthood, placing untreated dental caries in the permanent dentition as the most prevalent disease at 35% of the population. For our actions to disrupt the demineralization process and to have a meaningful impact on this burden of disease, we need to identify carious lesions early in their natural history, affording the best opportunity to prevent

progression from surface intact lesions to those requiring restorative treatment. In the absence of early intervention and treatment, caries sufferers experience pain, discomfort, and lack of function.

Wolff discusses the current methods and therapeutics that are used to shift the biological balance towards remineralization or at least arrest the carious process.[2] His review covers fluoride, both in its familiar forms (gels, varnishes, and dentifrices) and as silver diamine fluoride (SDF). It is interesting to note that the silver element of the SDF complex is thought to exert an antimicrobial effect, inhibiting *Streptococcus mutans*[A1] [A2] and *Lactobacillus acidophilus*. The move to consider the microbiome in the caries process is the subject of the next paper.

Oral Microbiome and Arginine: Mechanisms of Action and Laboratory Studies—Burne and Shi

Dr. Robert A. Burne et al.[3] provides us with a tour-de-force review of the role of the microbiome in dental disease, with an important statement that helps us understand how new therapeutics may fit within the framework of disease management described by Pitts and Wolff:

> Present therapeutic and treatment strategies for caries largely ignore the critical role that intermicrobial interactions and microbial ecology play in the development and progression of the disease. Vaccination has fallen by the wayside, while biofilm removal and excavation of damaged tissue have remained the standard of care, although major strides have been made in the nonsurgical management of caries. Recently, though, basic and clinical research has highlighted that incorporation of arginine into oral health care formulations is a promising novel approach to caries prevention and management.

What is arginine? Arginine is an amino acid that was first isolated in 1886 from lupin seedlings and is classified as a conditionally (or semi-) essential amino acid and one that is sold in numerous food supplements. Its ability to impact the oral microbiome (and hence caries) was recognized as early as 1970, but it is only recently that its mechanism of action has become fully understood. Burne states that the primary actions of arginine are dependent on the biochemical and physiological activities of the oral microbiome[3] and goes on to describe how arginine provides selective bioenergetic advantages to health-associated microbiota, pushing the microbiome in essence towards favoring remineralization. He describes in vitro and in vivo studies of arginine that suggest it can be a "highly effective anti-caries agent on its own, or in combination with fluoride".[3] Arginine improves the stability of plaque pH, inhibits the actions of cariogenic pathogens, and favors the microbiome towards a health-promoting profile.

This leads us to the paper by Dr. Wenyuan Shi et al.[4] that further explores the mechanisms by which arginine exerts its anticaries effects, examining and describing studies that show how oral bacteria metabolize arginine and, in turn, how this improves pH homeostasis, modulates the microbial profile, and inhibits caries pathogens. Shi describes arginine as a prebiotic—a substrate that is selectively used by microorganisms to promote or confer a health benefit. Shi concludes that the evidence to support the use of arginine has largely been developed from in vitro and in vivo studies; hence, there is a need to supplement these findings within clinical studies.

Clinical Studies of Arginine—Ryan

Shi's conclusion leads us to the final paper in this special monograph, where Dr. Maria Ryan[5] describes the current clinical studies that have examined the use of arginine as a caries therapeutic. She describes promising results from studies where arginine has been used with fluoride and alone, with the potential for arginine-only toothpastes to benefit those areas of the world where fluorosis remains an issue, or where, for various cultural reasons, there is a low uptake of fluoride-containing dentifrices, leaving consumers with the choice of a toothpaste with no active anticaries ingredient.

As an example of the studies described, a 2-year double-blind randomized control trial found that a dentifrice with 1.5% arginine was at least as effective as a 1100 ppm fluoride toothpaste in reducing the formation of caries.[6] Ryan describes a developing portfolio of clinical evidence that supports the efficacy of arginine as a novel caries preventive agent.

CONCLUSION

The clinical studies described by Ryan[5] lend further weight to the developing evidence that has been so well articulated by the contributors to this special monograph—that arginine is a promising anticaries agent with a sound biological mechanism of action that may offer, for the first time in decades, a new caries therapeutic.

There are currently further large-scale clinical studies being undertaken both in China and the United States and, as a caries researcher, it is exciting to see that this area of study has been invigorated by the potential for a new dentifrice formulation. I invite you to read each of the scholarly articles in this special monograph; they are a superb resource not only on a novel therapeutic but for the underlying basis of the oral disease caries known to be prevalent throughout the world that, as dentists, we spend a great amount of our time treating and trying to prevent.

Conflict of Interest Statement

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CARIES OVERVIEW 2021: EPIDEMIOLOGY, ETIOLOGY, AND EVIDENCE-INTO-ACTION CONSIDERATIONS

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ABSTRACT

Background: This report provides an overview of dental caries in 2021, with an emphasis on how current research on epidemiology, etiology, and early detection fits with evidence-into-action initiatives across multiple stakeholder domains.
Methods: This overview, based on recent reviews and reports from the international literature, highlights key shifts in thinking about caries and explores related implementation and policy activities pertaining to caries classification and management.
Results: It is important to understand the scale of untreated caries as a continuing global problem for adults and children, as recognized in the 2021 World Health Organization resolution on oral health. Changes in our understanding of the caries process and its interaction with the oral microbiome have led to an appreciation of caries as a noncommunicable disease and enable caries control by maintaining the ecological balance between health and disease. Early detection of caries is now seen as a vital component of caries assessments in both public health and clinical practice. The evolution of integrated caries classification and management systems has led to a focus on a holistic, patient-centered, long-term view of caries management, monitoring, and outcomes. These findings have informed a range of evidence-into-action activities that have the potential to improve health and treatment in a post–COVID-19 world, as we move toward making cavities history.
Conclusions: Integration of new and existing concepts of caries offers novel ways in which prevention can be achieved and care provided for populations and individuals to maximize health outcomes while minimizing surgical intervention and aerosol generation.
Practical Implications: Improved understanding of the dynamics of the dental caries process, together with enhanced understanding of how to manage risk and create a balance between health and disease have led to the development of practical "4D" tools to help prevent and control caries in clinical practice with a minimally interventive approach associated with less aerosol generation.

BACKGROUND AND METHODS

As a prelude to a series of more focused articles, this report provides an overview of dental caries in 2021, with particular emphasis on how current research evidence on epidemiology, etiology, and early detection fits with recent international evidence-into-action initiatives taking place across multiple stakeholder domains.

This overview, based on peer-reviewed articles and reviews drawn from the international literature, highlights key shifts in thinking about caries, ranging from terminology to disease concepts, care planning, and public health implications. It also explores recent implementation and policy activities pertaining to caries classification and management and highlights how the COVID-19 pandemic is now driving the adoption of minimally interventive caries management and prevention linked to the first World Health Organization (WHO) oral health resolution in 14 years.

Evolution of terminology and concepts

Repeated discussions among researchers, clinicians, public health professionals, and policy makers regarding the most appropriate terms to use to describe conditions and characteristics of dental caries and related matters prompted the European Organisation for Caries Research (ORCA) and the International Association for Dental Research (IADR) Cariology Research Group to hold a joint workshop in 2019 in Frankfurt, Germany.[1] Workshop participants selected commonly used terms and reviewed their definitions, based on current concepts and science, and then agreed by consensus (through blind voting) on the most appropriate terms and definitions. The definition of "dental caries as a disease" (with 100% agreement) was the following[1]:

> Dental caries is a biofilm-mediated, diet modulated, multifactorial, non-communicable, dynamic disease resulting in net mineral loss of dental hard tissues . . . . It is determined by biological, behavioral, psychosocial, and environmental factors. As a consequence of this process, a caries lesion develops.

The definition of "caries care/management/control" (again with 100% agreement) was this[1]:

> Caries care/management/control are actions taken to interfere with mineral loss at all stages of the caries disease . . ., including non-operative and operative interventions/treatments. Because of the continuous de/remineralization processes, caries control needs to be continued throughout the life course. The terms caries care/management/control may be more appropriate than the term caries prevention.

The definition of "caries prevention" (with 88% agreement) was as follows[1]:

> Caries prevention traditionally meant inhibition of caries initiation, otherwise called primary prevention. Primary, together with secondary and tertiary prevention, comprising non-operative and operative treatments, are now summarized under Caries care/management/control.

These definitions are based on both current concepts in the scientific literature and on experts' opinions. The IADR and ORCA groups stated that the "suggested terminology is recommended for the use in research, in the public health field, as well as in clinical practice."[1] Such terminology and the concepts behind the definitions are markedly different from those taught to researchers, clinicians, and dental public health staff in earlier decades and represent a challenge to many stakeholder groups.

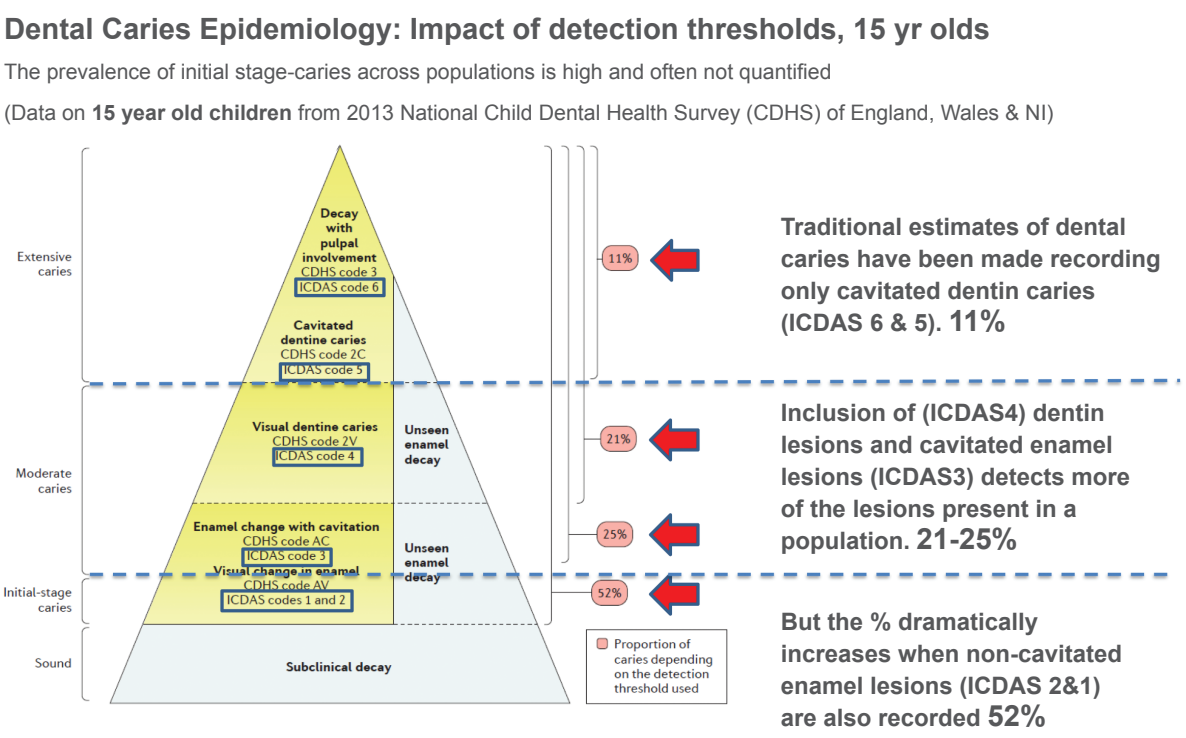
RESULTS

Epidemiology

The evidence base on caries detection and assessment has developed over many decades and the current concept of including clinical visual information on both enamel and dentin caries detection is not new.[2-4] Key findings were published in the 1950s, 1960s, and 1970s, but there has been, in many countries, a failure to understand or implement these research findings. The newer guidance regarding epidemiology and clinical practice is, in fact, based on many decades of research.

Unfortunately, there are serious challenges associated with maintaining robust and reliable estimates of caries prevalence internationally, as well as understanding estimates of preventive and restorative care needs. These challenges stem from a limited understanding of the impact of caries detection thresholds in dental caries epidemiology. Figure 1 shows the proportion of 15-year-old

Figure 1



Pitts NB, Zero D, Marsh P, Ekstrand K, Weintraub J, Ramos-Gomez J, Tagami J, Twetman S, Tsakos G and Ismail A. Dental caries. Nat. Rev. Dis. Primers 3, 17030 (2017).

adolescents from a 2013 survey of child dental health with dental caries at a range of detection cutoffs.[5,6] These thresholds are based on the International Caries Detection and Assessment System (ICDAS) criteria,[7-9] which range from codes 1 and 2 (initial-stage caries) to codes 3 and 4 (moderate caries) and codes 5 and 6 (extensive caries). These are the same thresholds used in the American Dental Association Caries Classification System.[10] Figure 1 shows that if only traditional estimates of dental caries are used (ie, only open cavitated dentin caries [ICDAS 5 and 6]), 11% of the adolescents are considered to have had caries. However, if dentin lesions with shadows under the enamel (ICDAS 4) and cavitated enamel lesions (ICDAS 3) are included, 21% to 25% of the population is considered to have had caries. If the clinically relevant and potentially reversible initial stage lesions are included (including the noncavitated enamel lesions [ICDAS 1 and 2] found on examination in the field), then the percentage of adolescents with caries dramatically increases to 52%.[5,6] Collection of caries data using this ICDAS-based system allows greater insights for both epidemiology and clinical practice[11]; it also enables the identification of clustering to inform population caries prevention strategies.[12]

The significant variations associated with different surveys in different countries using different caries detection thresholds (let alone variations in sampling methods and training and calibration of examiners) compromises our ability to make international or regional comparisons. This has been demonstrated in many parts of the world, including Europe[13] and Latin America.

Figure 2 provides an overview of caries epidemiology on a global level. The classical WHO criteria, which is compatible with the National Institute of Dental and Craniofacial Research criteria, only includes caries at the dentin cavitation level; data using these criteria are now more rarely collected or updated in valid national samples. Therefore, it is more difficult to assess genuine geographic variations in caries levels. In any case, these types of surveys systematically underestimate the true disease prevalence when initial (noncavitated) lesions (ICDAS 1 and 2 stages) are included (Figure 1). To try and address these issues, 3 organizations in Europe agreed to support a "Brussels Statement" on caries epidemiology,[14] which sets out needs for modern criteria, together with the ability to compute backwards compatible data estimating what the prevalence of caries would have been if traditional criteria had been used. The statement advocates the inclusion of initial lesions scored by trained and calibrated examiners.[14]

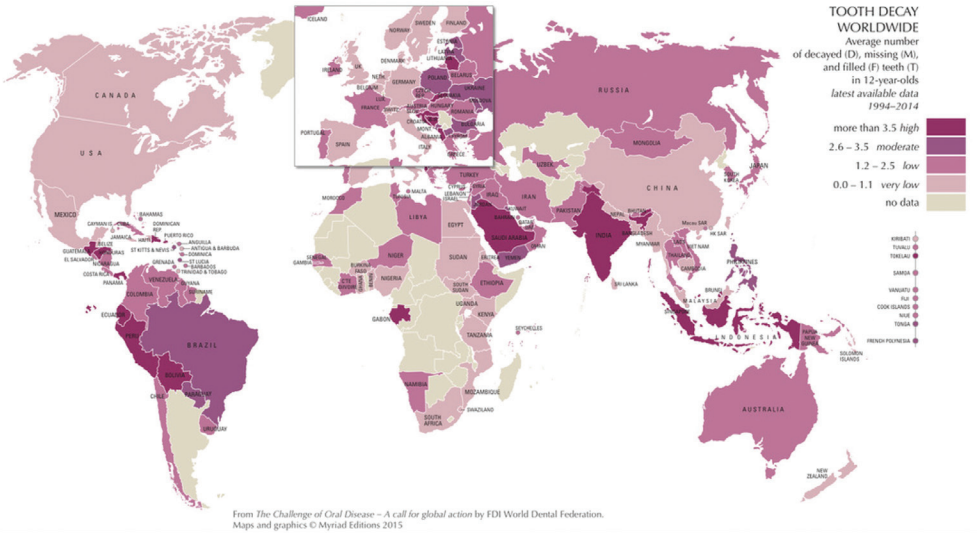
Over the past decade, inclusion of oral health data in the wider global burden of disease studies has provided a method of looking at caries prevalence based on the burden of caries as assessed by disability-adjusted life-years.[15] These data are increasingly important and used in policy and planning as measures of the disease according to its impact on individuals. Clearly, caries is a chronic disease that extends into adulthood[16]; untreated caries in the permanent dentition ranked No. 1 across all oral conditions, affecting 35.29% of individuals, and untreated caries in primary teeth ranked No. 10 across all oral conditions, affecting 9.02% of individuals. Therefore, it is important to understand the scale of untreated caries as a continuing global problem, with health, economic,[17] and social burdens for both adults and children. These issues have been explored in more depth through the Alliance for a Cavity-Free Future (ACFF) and its global consensus on policies to make cavities history.[18,19]

Figure 2

Overview of Caries: what we know and what we don't know

GCCM Global Collaboratory for Caries Management — King's College London and partners — working with ICDAS Foundation and ACFF

- **Classical "WHO"** (and **NIDCR**) "cavitation" data now more rarely collected/updated on valid National samples. These in any case systematically underestimate disease prevalence compared to when initial (non-cavitated) lesions at ICDAS 1 & 2 stages are also included (National Survey example from the "UK")
- **"Brussels Statement"** sets out needs for modern/backwards compatible Epidemiology, advocates inclusion of initial lesions
- **Global Burden of Disease Data** increasingly important and used, measures caries by its impact on individuals



Dental Caries: Global Burden Study - Prevalence of Oral Conditions

	Rank	N (in thousands)	%
Untreated caries permanent dentition	1	2431636	35.29
Severe periodontitis	6	743187	10.79
Untreated caries of deciduous teeth	10	621507	9.02
Severe tooth loss	36	158284	2.3

Oral conditions collectively affected 3.9 billion people

Marcenes, W. et al. Global burden of oral conditions in 1990–2010: a systematic analysis. J. Dent. Res.92, 592–597 (2013)

Selwitz R H, Ismail A I, Pitts N B. Dental caries. Lancet 2007; 369: 51-59.

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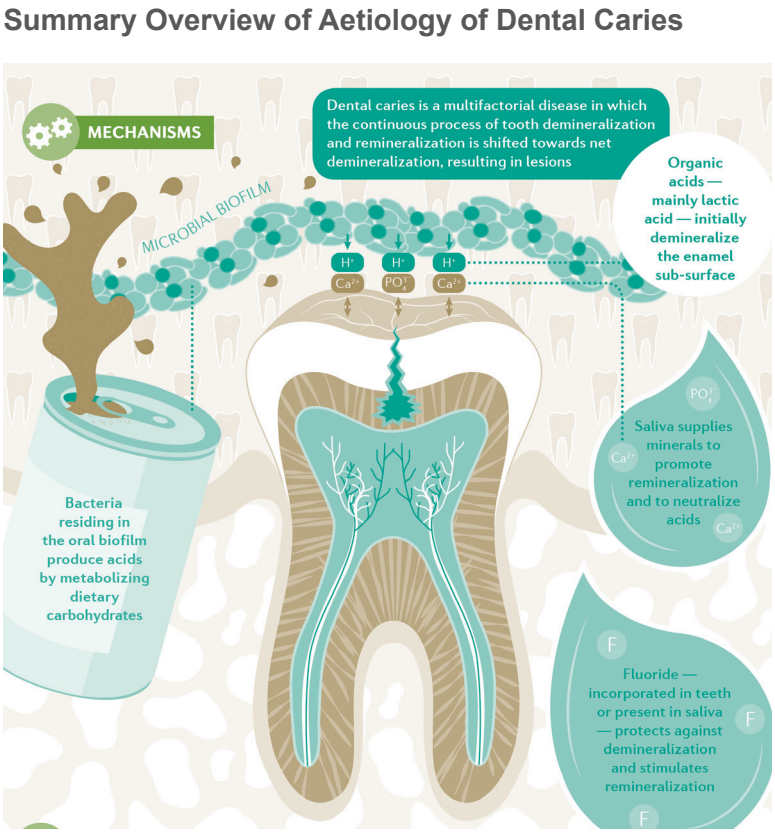
Etiology

The etiology of dental caries is complex, the understanding of which has evolved over the past 15 years.[1,2,4] A recent review of dental caries aimed at researchers across a range of scientific disciplines[4] generated a useful infographic, part of which is shown in Figure 3. This infographic demonstrates the interaction over time between the ecological community of bacteria at specific sites within the oral biofilm, producing organic acids by metabolizing dietary carbohydrates, and the tooth surface and subsurface on which results episodic demineralization and remineralization of the tooth, saliva, and any available fluoride. Our understanding of the caries process and its interaction with the oral biofilm/microbiome has changed over time. Rather than implicate specific organisms as the only way in which caries can be initiated or can progress, there is now an appreciation of the importance of maintaining a healthy environment at the tooth surface. Increased sugar intake, more frequent acid production, and changes in the pH at the tooth surface drive an ecological shift to a more pathogenic biofilm.[4] Knowing that the environment can drive the caries process offers new opportunities to intervene to maintain health. Moreover, dental caries is now considered to be a noncommunicable disease,[20] which has important policy implications.[18,19]

Consequently, in understanding and controlling dental caries, an increasing emphasis is being placed on balancing pathologic and protective factors. Figure 4 presents the pathologic factors that lead to demineralization and the balancing protective factors that lead to remineralization and lesion arrest or regression. This biological balance of factors has led to an enhanced appreciation of achieving caries control by maintaining the ecological balance between health and disease.

Figure 3



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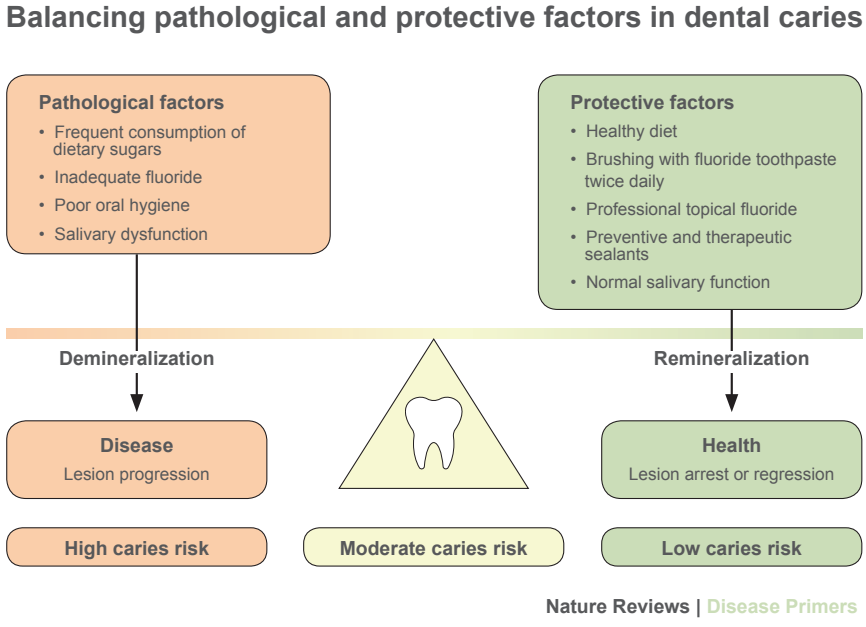
Detection, measurement, and monitoring of lesions

A complete description of the evolution of caries classification systems is beyond the scope of this report, but it has been documented elsewhere.[2-4,7-9,21,22] In summary, the ICDAS system was developed in 2002 "to produce a harmonised, evidence-based, international, system to lead to better quality information to inform decisions about appropriate diagnosis, prognosis and clinical management at both the individual and public health levels."[7] The system was developed in response to the widespread inability to compare results from different studies that used incompatible diagnostic criteria. From the start, ICDAS was designed to be applicable to epidemiology, clinical practice, research, and education. The system enables clinicians to detect lesions at various histologically and clinically meaningful stages, assess the activity of each lesion at a specific point in time, and then facilitate monitoring of lesions over time.[23]

To advance work in this field internationally, in 2013 the Global Collaboratory for Caries Management was formed as an umbrella group to facilitate delivery of preventive dental medicine by bringing together workstreams being undertaken by

Figure 4



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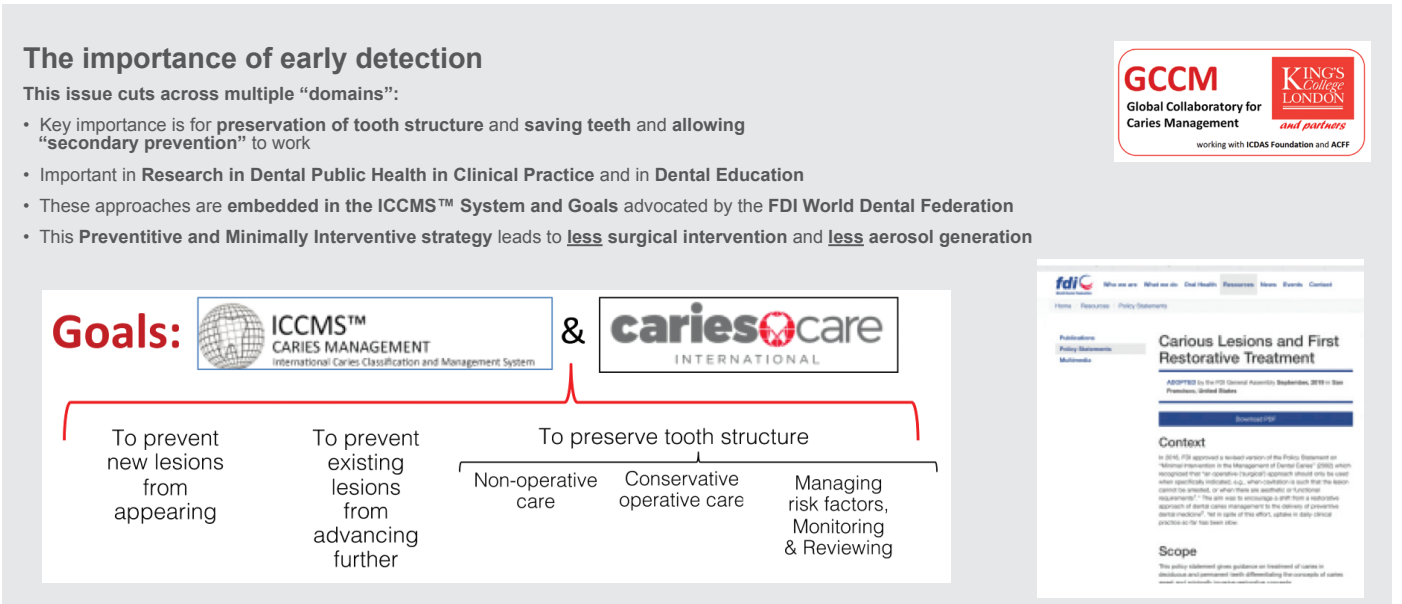
Nat. Rev. Dis. Primers doi: 10.1038/nrdp.2017.30

Figure 5



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King's College London, London, England, and 2 partners: the ICDAS Foundation and the ACFF. This undertaking has allowed ICDAS to lead the development of the International Caries Classification and Management System (ICCMS), which evolved through workshops held in several cities in Europe and the United States.[24] In parallel, the ACFF and King's College London led a series of 3 Dental Policy Labs that brought together disparate stakeholders to develop strategies for moving toward a cavity-free future.[25] The project encompasses health, economic, and policy considerations, as well as paying for health in dentistry,[26] and then moves toward oral and dental health through partnership[27] by linking dental public health, behavioral change, and industry. The 2 strands then converge, with the development of the tailored version of ICCMS for practice known as the CariesCare International "4D" system.[28,29] The strategy that emerged, with consensus from many contributors over 18 years, focuses on the preservation of tooth structure and allowing time for secondary prevention of caries to work. These integrated caries classification and management systems have led to a holistic, patient-centered, long-term view of caries control, monitoring, and outcomes.

Importance of early detection

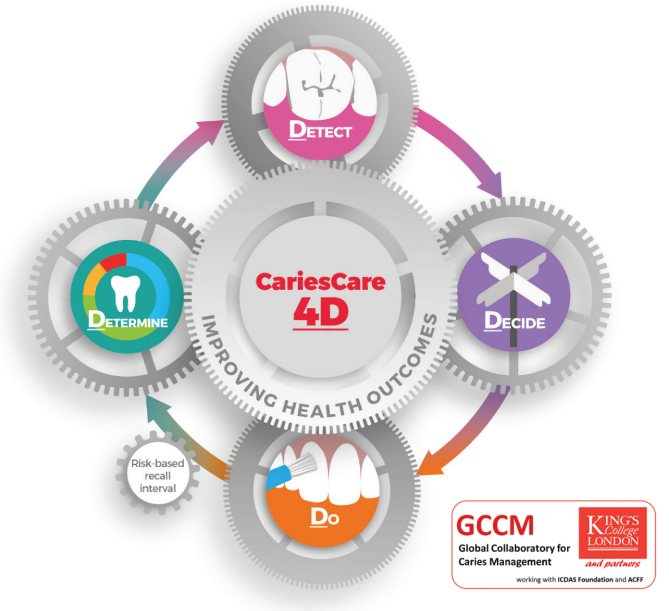
Early detection of caries is important across multiple domains and is now seen as a vital part of caries assessments in both public health settings and clinical practices. Figure 5 illustrates the importance of early detection in terms of preservation of tooth structure and saving teeth, as well as its value in research, dental public health, clinical practice, and dental education. These approaches are embedded in the ICCMS system (which is now advocated by the FDI World Dental Federation[30]) and its goals, which center on preventing new lesions from appearing, preventing existing lesions from advancing further, and preserving tooth structure.[24] The system and the approach used are also supported by a wider range of international evidence,[31-36] including that for the problematic field of early childhood caries.[37,38] In addition, and most importantly during the global COVID-19 pandemic, this preventive and minimally interventive strategy leads to fewer surgical interventions and consequently less aerosol generation.

Related evidence-into-action activities

These findings from caries research have informed a range of evidence-into-action activities across 5 stakeholder domains. These are further research,[4] clinical practice,[28,29] dental education,[39-41] public health,[14] and policy.[25-27] These activities have the potential to improve caries care in a post–COVID-19 world. To highlight

Figure 6

There is now international consensus on what needs to be implemented to use ICCMS™ in dental practice



2 examples, let us consider implementation of ICCMS in clinical practice and the impact of the Dental Policy Labs.

Figure 6 illustrates the CariesCare International 4D system,[28,29] which was developed by an international team to facilitate implementation of ICCMS into dental practice. The system aims to be practice-friendly and to guide the dental team and the patient through a 4-step cycle to improve health outcomes[42]:

1. Determining caries risk
2. Detecting and assessing each lesion
3. Deciding on a personalized care plan for each patient
4. Doing minimally interventive tooth-preserving care before using a risk-based recall to start the cycle again.

This 4D system is supported by an international consensus on evidence into practice (endorsed by 44 authors from 17 countries)[28] and an illustrated case study.[29] In addition, there is a dedicated website (https://cariescareinternational.com/) with access to documents, translations, lectures, and e-learning resources.

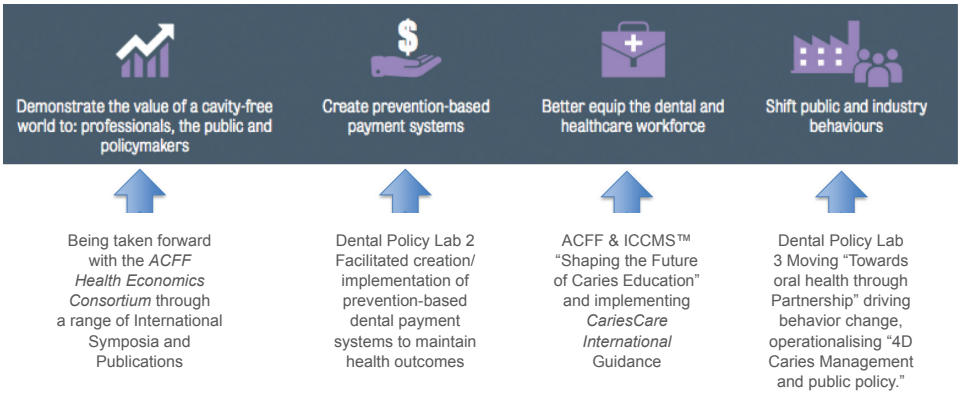
This work has generated 2 editorials in the *British Dental Journal*. In the first editorial, Hancocks[43] supports the move to more preventive and less invasive care, argues that it should be paid for, and links the approach to the implementation of the United Nations Environment Programme International Minamata agreement, which seeks a global reduction in the use of mercury and consequently the international phase down of the use of dental amalgam. The author suggests that CariesCare could lead the way toward "minimal intervention dentistry" globally. The second editorial by Hancocks[44] was published shortly after the international lockdown associated with the COVID-19 pandemic. He somewhat bravely explored inconsistencies in the attitudes with which the public and profession view health and disease, looked at the burdens associated with untreated dental caries, and contrasted them with both coronavirus and contaminated water. Citing a Dental Policy Lab meeting[27] and the CariesCare International 4D system,[28] Hancocks opined that the recent dramatic global changes in behavior due to the pandemic may lead to a situation in which stakeholders such as governments, societies, populations, and individuals can work collaboratively to control caries. For at least 17 years, the dental community has been asking itself if we are ready to move from operative to nonoperative/preventive treatment of dental caries in clinical practice,[45] and the COVID-19 pandemic just may provide the stimulus for widespread change.

In a guest editorial, Klemmedson[46] pointed out that the COVID-19 pandemic has led to a "renewed opportunity to emphasize prevention and to promote indications for noninvasive caries management." In addition, during the pandemic, an international group adapted CariesCare International protocols for use in children. It shared knowledge and experiences across countries and centers using teledentistry and a range of nonaerosol-generating procedures and published a 1-year "Caries OUT" multicenter single-group interventional study protocol.[47]

Figure 7 summarizes the significant progress being made in getting evidence into action through the ACFF/King's College London Dental Policy Labs. The 4 priorities identified by the first Dental Policy Lab[25] are being advanced internationally. These actions are to (1) better demonstrate the overall and economic value of a cavity-free future, (2) create prevention-based payment systems, (3) better equip the dental and health care workforce, and (4) shift public and industry behaviors. The lab participants, who come from many diverse stakeholder groups, developed recommendations that have had a substantial impact, and the ACFF/King's College London Oral Health Policy Lab Network is currently being established to allow implementation experiences to be shared and the wide range of key recommendations to be advanced.[25-27] In addition, the Making Cavities History global consensus policies[18,19] facilitated by ACFF can now be used globally and locally to help implement the 2021 recommendations of the WHO resolution on oral health, the first to be issued in 14 years.[48]

Figure 7 **Significant progress is being made on getting Evidence into Action through the ACFF/King's College London "Dental Policy Labs"**

The **four policy priorities** identified by the first Dental Policy Labs are all being advanced internationally



CONCLUSIONS

Integration of new and existing concepts of caries affects how we refer to it, detect it, and prevent and control it. These concepts also impact our assessment of how and when we should intervene surgically. At a time of dramatic change, these insights offer new ways in which caries prevention can be achieved and care can be provided for populations and individuals to maximize health outcomes and minimize both surgical intervention and generation of aerosols.

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- those who have joined with us from other organizations around the world;
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CARIES—AN ONGOING PUBLIC HEALTH CRISIS

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ABSTRACT

Despite being a preventable disease, dental caries remains a global health crisis. Our understanding of the caries process has changed over the last few years; it has evolved from being a unidirectional process resulting in cavitation to a dynamic process of demineralization and remineralization. An examination of the impact of caries as a global crisis demonstrates that caries continues to be a chronic problem despite being a preventable and treatable disease. The impact of caries is seen in the health and wellbeing of the population, as well as the economy. Current preventative methodologies have focused on the utilization of fluoride to increase the remineralization process and reduce demineralization. Fluoride has a wide range of applications, from at-home techniques such as fluoridated toothpaste and mouth rinses to community-level approaches such as public water fluoridation. Additional management methodologies have emerged; silver diamine fluoride has only recently been introduced in countries such as the United States despite being used for decades as a caries-arresting material in Asian countries such as Japan. The benefits of such a medicament have been crucial in approaching caries control in underserved populations as well as in aging and special health care needs patients. Dental technology continues to improve, and the latest focus has been on a new biofilm-modifying preventative alternative: arginine toothpaste. The incorporation of arginine in the biofilm is believed to create a probiotic/prebiotic effect in which commensal bacteria create a more alkaline environment, favoring a reduced acidic environment that causes tooth demineralization. Fortunately, once caries demineralization has extended beyond the stage at which remineralization can restore surface integrity, the carious tooth can still be managed without surgical restoration by caries arrest.

INTRODUCTION

To best understand the "ongoing public health crisis" of caries, it is necessary to discuss the evolving definition of dental caries. Although the current definition of dental caries is accurate, the localized destruction of susceptible dental hard tissue by acidic byproducts from bacterial fermentation of dietary carbohydrates,[1] this definition has expanded to a more complex discussion of the caries process that represents a continuum of tooth demineralization/remineralization. The "modern" view of dental caries started with W.D. Miller's (1890) definition of a 2-step process whereby bacteria on the tooth, exposed to fermentable carbohydrates, produce acid and in a second step dissolve the surface of the tooth.[2] Stephan demonstrated that this production of acid after exposure to the fermentable carbohydrate resulted in a localized drop in pH within the plaque, followed by a subsequent return to the baseline pH over time.[3,4] This set the stage for caries being a cyclic event of demineralization/neutrality/remineralization. Englander (1959) demonstrated the role of saliva in neutralizing the fall in plaque pH after exposure to fermentable carbohydrate.[5,6] Although considerable attention has been placed on a few bacterial species as the cause of dental caries (eg, *Streptococcus mutans, Lactobacillus*…), there is general agreement today that the dental biofilm exists as a complex ecosystem that can shift from a neutral pH to a more acidic

ecosystem.[7,8] Today, dental caries is accepted as an imbalance in the cyclic process of demineralization and remineralization of tooth structure. This is caused by a mixed ecology biofilm consuming fermentable carbohydrate excreting acids resulting in a pH maximal drop followed by the return of the pH to initial pH modulated by saliva. There is currently a recognition that the mixed ecology of the biofilm may be naturally shifted in composition to a more acidic ecology by repeated exposure to fermentable carbohydrate.[7,8] Saliva helps modulate both the composition of the biofilm[7] and the recovery of the biofilm pH after sugar challenge.[5] The susceptibility of the tooth to demineralization may be modulated by the incorporation of fluoride in the tooth structure.[9] Dental caries is a continuum that involves the balance between the time a tooth surface spends in demineralization versus the time remineralizing. An imbalance in the continuum resulting in greater time spent in demineralization will lead to tooth surface alterations that eventually result in cavitation. There are a number of items that influence the time a tooth spends in demineralization. The increase in frequency of sugar consumption and an increase in sugar retention time directly relate to an increase in demineralization of teeth resulting in cavitation, measured clinically as decay/missing/filled/teeth (DMFT),[10] but it must be remembered that in this balance, frequent/longer acid cycles result in a shift in the biofilm flora in favor of acidogenic bacteria.[8] Importantly, an increase in the quantity of sugar consumed alone is not a predictor of increased caries, as many communities around the world have increased caries preventive strategies over the same time period as sugar consumption has increased.[11] Decreases in saliva flow can also favor a shift in the caries balance toward demineralization, resulting in an increase in caries progression.[12,13] The caries balance is affected by multiple social determinants favoring demineralization that will be discussed later. Research has demonstrated that acid is not the only product of the mixed ecology. Alkali production has potential in changing the pH of the oral biofilm, which impacts demineralization.[14] Recent dental research has focused on the ability of an amino acid, arginine, within toothpaste to complement the anti-caries effect of fluoride. By including arginine in toothpaste as a prebiotic, microorganisms commensal in the biofilm create a more alkaline environment that helps to reduce the acidity that causes enamel demineralization and caries formation in the first place.[15]

Dental caries is a preventable disease, yet it consistently ranks as one of the most common diseases affecting the worldwide population.[16] One of the more evolutionary changes in the understanding of caries is that caries is a lifelong disease.[17] Increases in the number of untreated carious teeth are seen in an aging population worldwide as individuals live longer with their teeth. Although traditional dental care has focused on repairing decayed teeth to restore form and function, the last few decades have attempted to develop a more preventative approach to caries management. Fluoride has been shown to be one of the primary methods available to combat the effects of demineralization of teeth. Today, fluoride is utilized in community water, fluoride toothpaste, silver diamine fluoride, and high-concentration fluoride supplements to manage caries.

Caries as Global Health Problem

Dental caries remains a global health problem and is consistently recognized as a highly prevalent yet neglected health concern.[18] The Lancet Commission on Oral Health (2019),[19] a recent World Health Assembly Resolution,[20] and Vision 2030: Delivering Optimal Oral Health for All[21] each point to the ongoing nature of the global caries crisis. Across the globe, the number of people who have some type of untreated oral condition (primarily untreated caries, severe periodontitis, and severe tooth loss) has been on the rise. In the Global Burden of Disease 2017 review, 3.5 billion people worldwide have been afflicted by oral disease; of this number, 2.3 billion had some form of untreated dental decay.[22] Although caries was found to be ubiquitous throughout the world, the WHO Global Oral Data Bank survey of 12-year-olds found that reported prevalence ranged from 90% to 20%, with DMFT ranging from 0.3 to 6.1.[23] The disparity within countries and between countries was not always explained by socioeconomic status, access to care, and rural versus urban communities. The burden of tooth decay for 12-year-olds was highest in middle-income countries, with about two-thirds of decay remaining untreated.[24] A review of studies in the Africa and Middle East Region found that caries prevalence and DMFT was greater in urban versus rural for these countries, with the daily consumption of sugar in urban areas growing more rapidly than rural communities and the daily consumption of fruits greater for rural areas than urban in the same countries.[25] There has been a significant shift in burden of untreated caries globally, and, in high-income countries, the shift from children to adults challenges the convention that lower levels of caries in childhood will continue throughout a lifetime, with the concomitant cost of care rising steeply.[16,26]

The prevalence of caries and tooth loss due to caries in the United States has been essentially decreasing over the last half-century, but the data clearly demonstrate that there are social disparities in how well the United States is managing dental caries.[17] Unfortunately, there is a racial and ethnic disparity when evaluating oral health. The Surgeon General's report on Oral Health in America recognizes that caries is a disease of disparity, and it is important to acknowledge that it does not affect all Americans equally. According to the CDC, "Non-Hispanic blacks, Hispanics, and American Indians and Alaska Natives have the poorest oral health of any racial and ethnic groups in the United States."[13] Adults (35 to 44 years) who identify as Black, Non-Hispanic, and Mexican Americans have double the amount of untreated tooth decay compared with their non-Hispanic White counterparts. This disparity continues in Mexican American and non-Hispanic Black children having more tooth decay in the 3 to 5 and 6 to 9 age groups than their peers.[13] Based on these reports, we see that specific racial groups suffer a disproportionate burden for dental caries.

Dental caries also affect poor families to a greater extent. Children aged 5 to 19 from a low-income family (<100% of the federal poverty level) have at least double the amount of tooth decay compared with children from high-income families (>200% of the federal poverty level). Despite this, there is hope. Studies have evaluated poverty status and compared data from the periods of 1988 to 1994 and 1999 to 2004 demonstrating that although children from low-income families had more tooth decay in general, children from high-income families saw an increase in dental caries over the same time period, suggesting that the strategies used in low-income families, such as sealants, are helping the lower-income population.[17]

Economic and Social Impact of Dental Caries

In children, dental caries has been recognized as the single most common chronic disease. Dental caries is 5 times more common than asthma and affects 1 in 5 children in the United States.[13] Unfortunately, because of a lack of public awareness, dental caries remains a prevalent yet preventable disease, especially in this age group. Dental caries can have an impact on child development and learning. Pain from untreated decay can cause a child to lose the ability or desire to eat, resulting in deficient nutritional intake with consequent systemic effects.[27] Additionally, having pain or concerns

in the oral cavity can also affect a child's speech, self-esteem, and overall quality of life.[28] Children with active caries have been shown to miss more school days and, in general, perform worse than their peers.[13] These students also have more difficulty concentrating on their school work and tend to be more irritable because of their oral pain. It is estimated that every year, over 51 million hours of school are lost because of some dental-related cause.[29] Poor childhood caries control is a concern because caries of the primary dentition is a significant predictor of future decay in the permanent dentition. Combined with the fact that oral hygiene declines during the adolescent years, it is understandable that these patients will continue to be at high risk as they enter into adulthood.[30]

Adults actually experience more decay than children—1 in 4 adults have untreated decay.[29] Like children, adults miss a significant amount of time from work because of dental disease or visits. It is estimated adults miss 164 million hours a year of work because of oral health issues.[13] Dental emergencies are predicted to result in a $45 billion loss in productivity due to palliative or emergent care.[29] The high rate of decay in the overall population has also caused an increase in worldwide dental costs, which are estimated to reach over $544.41 billion US dollars in 2015.[31] Dental costs can be divided into 2 categories: Direct cost is the actual expenditure for care, whereas indirect cost is the loss in productivity due to disease. Regionally, high-income North America ranks at the top for direct costs around the world and is a close second for indirect costs. On an individual country level, the United States spends the most money for dental care and also loses the most money indirectly to this disease.[31] Considering the preventability of dental caries, there should be an increase in investment in the oral health of the American population.

Fluoride—Preventing Demineralization and Encouraging Remineralization

Fluoride, delivered through a variety of methods (community administered, home-administered fluoride toothpastes, and high-concentration fluorides), is a substantial factor in the reduction of caries burden over the last 75 years.[13] Although the systemic action of fluoride appears to yield significant protection when other sources of fluoride are not available, the topical effect of fluoride is recognized as being crucial. Toothbrushing alone can remove plaque from the tooth surface, preventing further demineralization, but the added effect of fluoride from toothpaste aids in the remineralization process, and fluoride can be incorporated into the tooth structure as fluorapatite, which builds resistance against an acidic environment. The introduction of a preventive brushing and fluoride program in the early 1980s in Germany decreased the number of teeth with decay in 12-year-olds by 93% and halved adult tooth loss between 1997 and 2014.[26]

Community fluoride administration includes milk, salt, and public water supply. Fluoride has also been incorporated into drinking water since 1945, when fluoride was first added to the water in Grand Rapids, Michigan; as of 2012, about 200 million people in the United States have benefitted from water fluoridation, especially those in underserved communities, those with disabilities, the aging, and those with economic challenges.[32,33] Water fluoridation has been acknowledged as one of the greatest public health achievements of the United States in the 20th century. It is safe, efficacious in preventing tooth decay, and generally considered an inexpensive cost per person for a community. One major negative health effect on the dentition associated with community water fluoridation and consumption of other sources of fluoride is fluorosis. Fluorosis may present in many different forms, ranging from the mild form in which white discolorations appear on the tooth surface to severe fluorosis in which the teeth can appear pitted or brown. The United States' population is at high risk for fluorosis because of the availability of multiple fluoride sources. The National Health and Nutrition Examination Survey data from 1999 to 2004 found 23% of Americans aged 6 to 49 were affected.[34] The majority of this population was afflicted by a mild form of fluorosis, and fewer than 1% experienced the severe form.

Fluoride-containing toothpastes have repeatedly demonstrated significant caries reductions if utilized once per day regardless of fluoridated water consumption, and greater improvements have been seen when utilized twice a day.[35] The use of additional topical fluoride, gels, rinses, and varnishes demonstrated additional caries preventive fraction.[36] Professionally applied fluoride varnish applications 2 to 4 times per year have demonstrated significant reductions in new caries for children and adolescents.[37,38] Fluoride has clearly played a significant role in the prevention of new dental caries over the last 6 to 7 decades.

Strategies for management of the biofilm for caries prevention

Management of the oral biofilm has been a cornerstone of dental health. Multiple investigations have been conducted to make the biofilm less cariogenic by regulating *S. mutans* through novel molecules, peptides, and sugars with little success in actual caries reductions.[39-43] Attempts to alter the biofilm microenvironment have offered hope for a new pathway to reduce caries. The naturally occurring amino acid arginine was found to be metabolized by a number of commensal bacteria such as *Streptococcus sanguinis* in the biofilm and produce ammonia, which is basic.[44-47] Studies have demonstrated that patients brushing with a 1.5% arginine-containing toothpaste develop higher resting plaque pH and a plaque that does not have as low a terminal pH when challenged by sugar. This decreases the interval below the critical pH that most consider the time spent demineralizing the tooth surface. This higher pH is primarily the effect of arginine metabolism and the production of ammonia, as well as lower levels of lactic acid.[45,47] Multiple clinical studies have demonstrated improved increment of caries prevention and early caries lesion remineralization.[47-50] These clinical studies demonstrate both a prevention of new decay and remineralization of early lesions utilizing quantitative light-induced fluorescence, as well as reduction of DMFT in long-term clinical trials.

Management of the caries lesion

Restoration of teeth does not treat the caries process. The need for prevention, early diagnosis, and management in the early stages of caries is critical.[51] Early noncavitated lesions may be treated utilizing highly conservative nonsurgical techniques that restore the tooth to health. Early lesions are considered those with intact or nearly intact enamel surface, where demineralization may be evidenced penetrating as far as the dentin. These lesions may be inactive, with a glossy smooth surface, or active, with a frosty roughened surface. Where inactive lesions may be observed for future changes in surface characteristics, active lesions require intervention both in risk reduction and in the attempt to remineralize.

Remineralization/Arresting Caries

Remineralization is the process by which the tooth gains calcium into the enamel and/or dentin that had previously been demineralized in the caries process. Remineralizing an active

caries lesion increases the mineral content of the tooth previously damaged by decay and, when occurring in a noncavitated early lesion, may actually restore the tooth's surface to a smooth surface. Arresting the caries process results in no further net loss of mineral from previously active caries lesions.[1] Essentially, arresting caries stops the biologic caries process from advancing and may or may not restore the tooth surface depending on the amount of pre-existing tooth destruction.

Two major therapeutics have emerged to manage the remineralization and/or arrest of caries: the utilization of fluoride on active caries lesions and the use of silver diamine fluoride (SDF). For noncavitated active lesions, sodium fluoride (5%) applied every 3 to 6 months has demonstrated the ability to arrest and remineralize coronal carious tooth structure, even without additional homecare or dietary modification.[52-54] Sodium fluoride (0.2%) mouth rinses utilized once per week and acidulated phosphate fluoride gel (1.23%) professionally applied 4 times per year have both demonstrated moderate certainty to arrest or reverse noncavitated lesions.[53] Cavitated lesions on all surfaces except root surfaces are less likely to arrest with the above fluoride alone than with SDF.[53] SDF, originally utilized in Japan in the 1960s to prevent caries, was officially introduced to the United States in 2014 as 38% SDF (with 5% fluoride) and as a means to reduce dentin sensitivity. Dentists have been using SDF (off label) to arrest carious lesions in the primary and permanent dentition. It is believed that the silver component of SDF has an antimicrobial effect, specifically inhibiting *S mutans* and *Lactobacillus acidophilus*, whereas fluoride is able to prevent further decay through remineralization because SDF is able to form CaF^2, which leads to the formation of fluorapatite.[55-57] Application of SDF is a quick procedure and easily completed with simple cotton roll isolation. SDF has been demonstrated to be highly effective in arresting decay and can penetrate the enamel to a depth of 25 µm and the dentin to 50 to 200 µm.[58] With regular applications twice a year, active caries are arrested and the incidence of new caries is reduced.[55] Biannual utilization of 38% SDF has demonstrated a 74% caries arrest rate.[59] When compared with fluoride varnish or glass ionomer, SDF is significantly more effective.[53,60] The concerns for utilization of SDF have primarily been regarding the side effect of tooth discoloration. Any decalcification or carious lesions may turn a dark brown or black color because of the silver phosphate being photosensitive.[59] In a study assessing parents' perception of SDF treated teeth, the majority of parents were not concerned by the discoloration to their children's teeth.[61,62]

Despite the success of SDF in arresting carious lesions, SDF does not rebuild tooth structure in gross lesions. Other techniques, such as the atraumatic restorative treatment (ART), may be utilized in conjunction with SDF to form the silver-modified ART or an ART restoration on its own. ART is a technique that can be performed without local anesthetic or rotary instruments, removing soft caries, finding caries-free margins, and placing a glass ionomer restoration to restore the tooth surface.[63] Adding a glass ionomer or resin-based glass ionomer over a frank cavitation protects the surface of the lesion and restores form; however, this technique is more successful for class 1 lesions than class 2 lesions.[64] ART restorations alone have demonstrated the ability to arrest caries and have similar long-term performance as amalgam restorations.[65]

Other methods, such as resin infiltration on smooth surfaces and sealants in pits and fissures, have demonstrated the ability to arrest cavitated and noncavitated dental caries, frequently in concert with 5% sodium fluoride varnish, although the evidence supporting the use of both therapies has a low certainty.[54] There is also weak evidence for the effectiveness of 1% chlorhexidine/1% thymol varnish on arresting root caries.[53,66] SDF continues to be more successful in arresting decay and has seen increased utilization across the United States.

CONCLUSION

Dental caries continues to be a globally widespread disease, creating a significant financial and social burden on a worldwide scale. Our understanding of the dental disease has been refined over the years. As we learn more about the dental biofilm and its characteristics, dentistry is able to develop new methods to disrupt the biofilm, prevent initial caries, or arrest and reverse current lesions.

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ANTI-CARIES MECHANISMS OF ACTION OF ARGININE

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ABSTRACT

Background: The potential for arginine to impact the oral microbiome and caries was recognized in the 1970s. Over the ensuing decades much insight was gained on how arginine was metabolized by the oral microbiome. More recent clinical and laboratory-based studies revealed potent anticaries activities of arginine and began to elucidate the mechanisms by which arginine may impact caries development (13-18). This paper summarizes salient findings related to metabolism of arginine by the oral microbiome in the context of dental caries.
Key Considerations: The primary mechanisms of action of arginine are almost entirely dependent on the biochemical and physiologic activities of the oral microbiome. In vitro and in vivo studies of the effects of arginine on caries and the microbiome demonstrate that arginine can be a highly effective anticaries agent on its own, or in combination with fluoride. As scientists unravel the intricacies of the oral microbial ecology, we are likely to continue to discover new ways in which arginine can influence biofilm ecology and health. Given the combination of the clinical successes with arginine to date, the growing demand for noninvasive prevention and treatment approaches for caries, and the potential that arginine can impact other diseases (periodontal diseases), respiratory infections and overall host responses by promoting the persistence of beneficial organisms in oral biofilms, research must continue apace to create a thorough understanding of what are likely many different mechanisms of action of arginine on human health.

BACKGROUND

The most common oral infectious diseases, dental caries and periodontitis, affect roughly half of the world's population and consume tremendous resources for prevention and treatment.[1] While these 2 infectious diseases have some very different characteristics, they share a common feature when viewed from the perspective of the oral microbiota: Both dental caries and periodontitis are ecologically driven diseases.[2-4] The oral microbiomes associated with healthy sites in the mouth differ in microbial composition and biological activities from diseased sites. In the case of caries, health-associated sites are dominated by commensal streptococci, such as *Streptococcus sanguinis* and *Streptococcus gordonii*.[5] This is also the case for a healthy periodontium, although there are some important distinctions between supra- and subgingival microbiomes.[6-7] Initiation and progression of a carious lesion is driven almost entirely by fermentation to acidic end products by the oral microbiota of carbohydrates ingested repeatedly by the host. The repeated acidification of tooth biofilms dissolves tooth mineral but also selects for a microbial population that is better able to grow and to metabolize sugars at lower pH values than health-associated microbes—a characteristic known as aciduricity. As carious lesions worsen, the proportions of highly aciduric microbes—*S mutans*, *Lactobacillus* spp., *Scardovia* spp., and others—continue to increase at the expense of many health-associated species.[5] Likewise, in periodontal diseases, the progression of a lesion is accompanied by

a decrease in the proportions of many gram-positive, sugar-utilizing bacteria and their replacement with strongly proteolytic gram-negative bacteria that can undermine host defenses and elicit tissue damage with a wide variety of biological molecules.[6,7]

Research conducted by basic, translational, and clinical scientists over the years has focused primarily on the organisms that show the strongest association with disease, referred to as pathogens, opportunistic pathogens, or pathobionts. Collectively, these studies have yielded a much better understanding of how the organisms associated with active disease—heretofore referred to simply as "pathogens"—establish, persist, and initiate or worsen disease. In contrast, the amount of research conducted on health-associated organisms, even those that are the most abundant species in oral biofilms, is only a small fraction of that focused on pathogens. Moreover, a substantial portion of the studies on the health-associated oral organisms have been focused on the role of these microbes in extraoral infectious diseases, eg, viridans streptococci in endocarditis. This asymmetry in knowledge was created not because there was a lack of recognition that the health-associated organisms might be doing things that are beneficial; for example, see reference 8. Rather, it was primarily attributable to logically organized priorities of clinicians, scientists, and funding agencies—*why would you not most vigorously study the organisms that are present when there is evidence of disease?* Fortunately, as DNA sequencing and other technologies have rapidly advanced, the foundation and rationale for devoting more effort to understanding the probiotic effects of potentially beneficial bacteria—ie, how health-associated bacteria may promote a healthy mouth and body—has been greatly strengthened. And, in concert with mechanistic studies, it is becoming clear that many of the health-associated commensal bacteria actively engage in modifying oral biofilm environments to make them more compatible with maintaining the integrity of the adjacent tissues and less favorable for the establishment or outgrowth of pathogenic organisms. While the statements in this paragraph apply to both caries and periodontal diseases, the remainder of this treatise will focus on dental caries.

Present therapeutic and treatment strategies for caries largely ignore the critical role that intermicrobial interactions and microbial ecology play in the development and progression of the disease. Vaccination has fallen by the wayside, while biofilm removal and excavation of damaged tissue have remained the standard of care, although major strides have been made in the nonsurgical management of caries. Recently, though, basic and clinical research has highlighted that incorporation of arginine into oral health care formulations is a promising novel approach to caries prevention and management.

The potential for arginine to impact the oral microbiome and caries was recognized in the 1970s. In the early 1980s, Kleinberg and colleagues demonstrated that arginine, as opposed to the other 19 biologically common amino acids, could serve particularly well as a substrate for mixtures of the oral microbiota from human subjects to generate ammonia (p*Ka* = 9.2)[9-12], as well as carbon dioxide. Specifically, when saliva was collected from volunteers and the bacteria were exposed to arginine or small arginine-containing peptides, a rapid rise in the pH was evident. Concomitant metabolism of arginine and sugar blunted acidification of the mixtures, whereas mixtures provided only with glucose rapidly reduced the pH. Over the ensuing decades, much insight was gained into how arginine was metabolized by the oral microbiome. More recent clinical and laboratory-based studies revealed potent anti-caries activities of arginine and began to elucidate the mechanisms by which arginine may impact caries development.[13-18] Here, we summarize salient findings related to metabolism of arginine by the oral microbiome in the context of dental caries.

Mechanism of action of arginine against caries

If there is only one take-away from this article (hopefully not), it should be that the primary mechanisms of action of arginine are almost entirely dependent on the biochemical and physiologic activities of the oral microbiome. That is not to say that arginine may not possess certain physicochemical properties that could allow the amino acid itself to interact with other factors on its own to influence disease development. However, from what is known at this time, such effects are probably minor compared to the impact that the bacterial metabolism of arginine has on caries formation.

When arginine is presented to oral biofilms, the primary route of metabolism is via a 3-enzyme pathway called the arginine deiminase system (ADS), which is not the same as amino acid deamination that is carried out by a broad spectrum of organisms.[8] Arginine is transported into the cell by ADS-positive bacteria, where it is acted

Figure 1

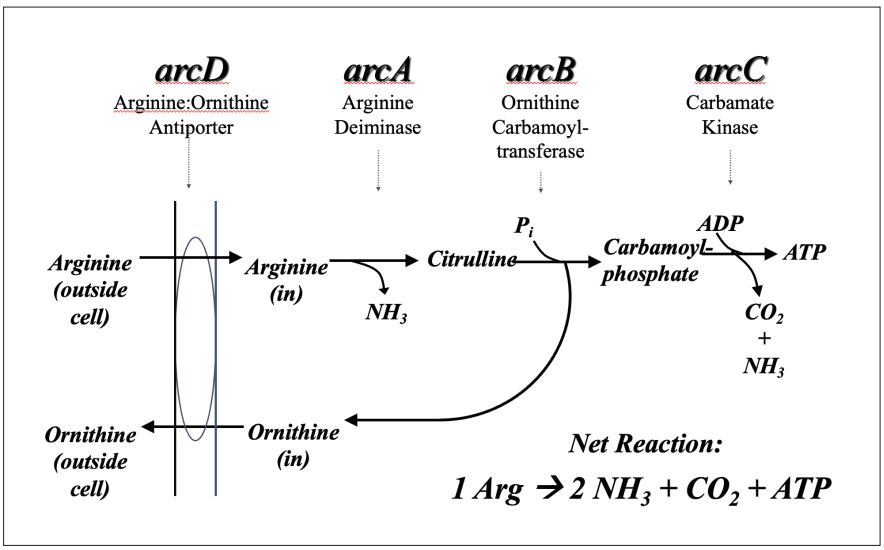

Figure 1. *The Arginine Deiminase Pathway. The core arginine deiminase system is a 3-enzyme pathway, encoded by the arcABC genes, that converts arginine to ornithine plus 2 molecules of NH_3 and one of CO_2. The final step, catalyzed by carbamate kinase, uses ADP to produce ATP. The ornithine created can be exchanged for arginine outside the cell by the ArcD arginine:ornithine antiporter with little bioenergetic cost, compared, for example, to transporters that internalize compounds at the expense of ATP. Many ADS-positive bacteria also have genes for cleaving arginine from peptides and for regulating the genes in response to arginine and oxygen.*

Figure 2

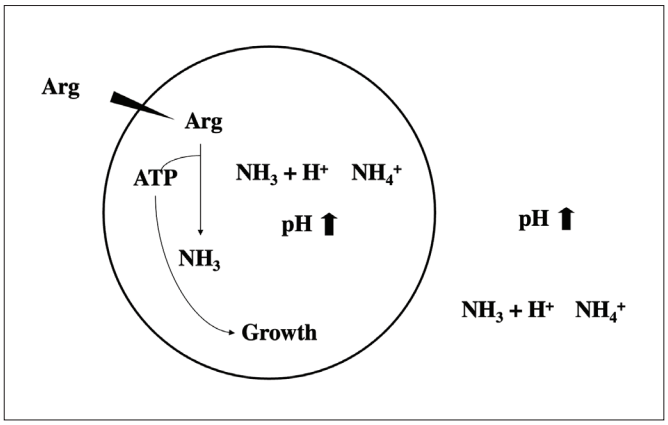

Figure 2. *Bioenergetics of Arginine Metabolism by the ADS. Many bacteria, oral streptococci in particular, will use ATP to move protons out of the cell to maintain an internal pH that is higher than the surroundings. The ammonia released by arginine inside the cell can raise the internal (cytoplasmic) pH of the cells, saving the cells tremendous amounts of ATP to maintain ΔpH (pH_{in} > pH_{out}). NH_3 can also diffuse rapidly through the cell membrane—it is uncharged—and raise the environmental pH. In addition, the ATP generated by arginine catabolism by the ADS can be used for growth and persistence. Thus, the ADS provides beneficial commensals with a substantial advantage when competing against caries pathogens.*

on by the enzyme arginine deiminase (ArcA) to yield 1 molecule of ammonia and 1 of citrulline (Fig. 1). The citrulline is acted on by a catabolic ornithine transcarbamylase to produce carbamyl phosphate and ornithine. While ornithine is usually excreted from the cells, the carbamyl phosphate is cleaved in the presence of ADP to yield 1 molecule each of CO_2 and ammonia, along with generation of ATP. Use of arginine by the ADS has multiple consequences that are directly related to its anticariogenic potential. First, the ammonia that is released plays two major roles. First, ammonia (uncharged NH_3) can rapidly diffuse through the cell membrane and into the surrounding biofilm matrix (Fig. 2). NH_3 rapidly equilibrates to ammonium ion (NH_4+) by complexing with a proton (H+), which raises the environmental pH. The increased pH does two important things. First, it reduces the tooth demineralizing potential of the biofilms and enhances the remineralization potential. Secondly, the elevated pH creates a local environment that is more favorable for the less-aciduric commensals and less favorable for aciduric caries pathogens, which use their acid tolerance to outcompete health-associated bacteria at low pH. Furthermore, the ammonia released inside ADS-positive bacteria confers a bioenergetic advantage to these microbes. In particular, bacterial cells actively strive to maintain an internal (cytoplasmic) pH that is higher than the surroundings when confronted with acidic environments. A major strategy used by the streptococci is to spend ATP to pump protons (H+) out of the cell to maintain the ΔpH component of the proton motive force (pH_{in} > pH_{out}). Each molecule of ammonia that consumes a proton in the cell saves the cell from having to move that proton out by using ATP; the ATP can be redirected to biosynthetic processes and growth. And, of course, the final step in the ADS pathway yields ATP, which offers the organism a direct bioenergetic benefit (Fig. 2). Notably, caries pathogens routinely lack the ADS. For example, there are hundreds of strains of *S mutans* that have been examined biochemically or at the genome sequence, and none have the ADS genes or enzymes. Importantly, the pH and bioenergetic benefits of arginine metabolism should ultimately translate into favorable modification of oral biofilm ecology. Real-world evidence that this is the case is accumulating. By way of example, site-specific dental plaque from health subjects harbors significantly more AD activity and can generate substantially more ammonia from arginine than biofilms from carious tissues, and the more advanced the lesion, the lower the ADS activity.[13] Moreover, when the microbiomes of caries-active subjects are compared with those of healthy subjects—using a technique called principal component analysis—they are fundamentally different. However, when those caries-active subjects utilize an arginine-containing (fluoride-free) dentifrice for as little as 2 weeks, a shift in the composition of the microbiome away from caries-active characteristics toward a health-associated state is observed.[15]

Other important mechanisms of action of arginine in prevention of caries

Clearly, providing selective bioenergetic advantages to health-associated, and often overtly beneficial, members of the oral microbiota and creating a tooth biofilm environment that is less acidic are major contributions of arginine metabolism to the promotion of oral health and the inhibition of dental caries. As we delve deeper into the complexities of the interactions between health-associated and pathogenic members of the tooth microbiota, though, we are learning that the "biofilm battles"[19] that influence the balance between health and disease are far more complex than previously appreciated.

First, though, it is important to highlight a key characteristic of the most abundant members of the oral microbiome: Individual isolates differ dramatically from one another, both in gene content and in phenotypic behaviors. This was first investigated in a comprehensive and systematic manner when the genomes of more than 60 isolates of *S mutans* from around the globe were sequenced and the data were subjected to phylogenomic analyses.[20] Much was learned and continues to be learned from this endeavor, but the salient points here are that while most isolates of *S mutans* carry about 2000 genes, only about 1400 of those genes are present in every isolate—and if all the genes that are carried by the species *S mutans* are added up, the number likely exceeds 4000 at this point in time. So, every isolate of *S mutans* has the potential to carry about 600 genes that are not evenly distributed in the population. As importantly, phenotypic behaviors that are thought to be critical to virulence—including essential cariogenic properties like aciduricity, acidogenesis, exopolysaccharide production, and oxidative stress tolerance—differ widely between strains with different gene content.[21] This genomic and phenotypic heterogeneity makes it difficult to target *S mutans* specifically with novel therapeutics unless they are directed at traits that are essential to initiating or worsening caries and are conserved and essential across all isolates.

Relevant to this discussion is the more recent observation that the most abundant commensal streptococci, including those that display overtly beneficial properties (eg, ADS-positive and/or inhibiting the growth of *S mutans*), show a similar, if not greater, degree of genomic and phenotypic heterogeneity. In the case of the commensals and *S mutans*, this genomic diversity has been attributed to promiscuous lateral gene transfer, as these organisms can actively take up and incorporate DNA from their surroundings into their genetic repertoire.[22] The degree to which genomic heterogeneity impacts our ability to understand the output from studies of the oral microbiome is illustrated by the fact that for some oral streptococci, it is not even possible to assign them reliably to a particular species, even after the entire genome sequence is known [22], which is attributable to the extensive gene exchange and genomic rearrangements that have occurred in these organisms. And, as for *S mutans*, the genomic heterogeneity manifests in tremendous

phenotypic heterogeneity. Unfortunately, what we know now is that species that have been associated with oral health can vary widely in their capacity to express beneficial traits, such as producing alkali from arginine, creating hydrogen peroxide (H_2O_2) that can kill many pathogens, and interfering with the growth of *S mutans* in other ways. Importantly, this explains, to a degree, the difficulties that have been encountered when investigators attempt to make definitive interpretations about differences in the composition of health- and disease-associated microbiomes. For example, an individual with caries may have high levels of commensal streptococci that are typically associated with dental health, but the strain(s) with which they are colonized may lack robust probiotic properties or may even be overtly cariogenic. These challenges are now being addressed by studying large panels of isolates to understand how the presence and expression levels of particular genes can influence beneficial or pathogenic potential. Fascinating data is emerging about the internecine warfare in oral biofilms, but it turns out that arginine can influence individual bacteria and interbacterial competition in intriguing and apparently very significant ways beyond the primary MOAs described above.

Novel MOA of arginine

Direct effects on S mutans

Work from the laboratories of Rickard in Michigan and Koo in Pennsylvania explored how arginine may impact the physical characteristics of biofilms formed by *S mutans* using single- or mixed-species in vitro biofilm models.[23,24] The takeaway from these elegant studies is that arginine itself may affect *S mutans* in ways that destabilize the biofilms that are formed in the presence of sucrose. Measurements of the forces that hold biofilms together and sophisticated microscopic dissection of the architecture of the biofilms formed in the presence and absence of arginine reveal significant spatiotemporal changes. A growing body of literature supports that such changes may affect the cariogenic potential of oral biofilms and that such changes would translate to diminished caries in humans, although most of the evidence is indirect. One important observation, though, is that arginine disrupted the formation of highly acidic microenvironments within the biofilms, which are key to damaging the tooth. More recently, Koo and Whiteley collaborated to image cariogenic biofilms from humans and then modeled those biofilms in vitro to provide intriguing evidence that directly supports that the way in which *S mutans* organizes with commensal streptococci and other bacteria may influence local acidification and thus directly affect the degree to which enamel is damaged.[25] If, as it appears, arginine can disrupt these interactions, such impacts could represent a novel MOA for arginine against caries.

Concurrently, our laboratory used a somewhat different approach to explore the impact of arginine on *S mutans*. Using various in vitro models and high-throughput quantitative sequencing (RNA sequencing) of mRNA from *S mutans* cultured with or without 1.5% arginine, it was shown that arginine—though it cannot be catabolized via the ADS—decreases acid and oxidative stress tolerance, affects expression of the enzymes required for exopolysaccharide production, and changes the overall transcriptome of the organism in ways that may reduce its potential to colonize, persist, and cause caries.[26] The mechanisms by which this occurs are not fully understood, as is the case for the above-refenced biofilm studies, but arginine appears to have the potential to diminish the cariogenic potential of *S mutans* absent the presence of ADS-positive commensals by inducing significant alterations in the expression of a large panel of genes that contribute to the virulence of *S mutans*.

Figure 3

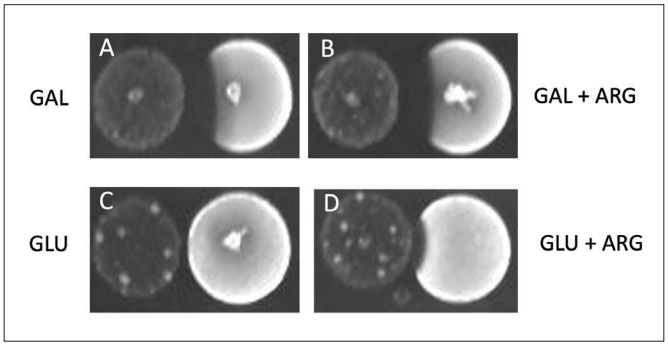

Figure 3. *Antagonism of S mutans by a Clinical Isolate of S mitis. Cells were cultured in broth, and a small aliquot of each culture was spotted onto rich medium agar plates containing galactose (A), galactose and 1.5 % arginine (B), glucose (C), or glucose and 1.5% arginine (D). In each panel, S mitis is on the left and S mutans is on the right. Without inhibition by S mutans, the bacterial colony would be a circle. Inhibition is evident, as there is a lack of growth of S mutans where it is closest to the S mitis colony.*

Impacts on interbacterial competition

Antagonism of one microorganism against others that are competing to persist in the same habitat is nearly universal, whether it is in soil, in water, on shower curtains, or in the human mouth. So is cooperation between microbes. In terms of arginine's impact on the ability of beneficial oral streptococci to interfere with growth and biological processes of *S mutans*, a fascinating story is developing. As noted above, there is a growing consensus that H_2O_2 production by commensal streptococci is an important ecological determinant, although the impacts may vary depending on the site in the mouth. In terms of caries, many commensal streptococci produce relatively high (millimolar) levels of H_2O_2. Whereas the commensals can tolerate such high levels of this compound, the growth and fitness of *S mutans* is compromised by much lower levels of H_2O_2. Thus, it has been posited that H_2O_2 production is a primary mechanism used by commensals in vivo in humans to discourage the establishment and reduce the cariogenicity of *S mutans*. As shown in Figure 3, H_2O_2 by a beneficial commensal is actually stimulated by the presence of arginine. Moreover, the increased production of H_2O_2 manifests in greater inhibition of *S mutans* by the commensal, and the enhancement in inhibition is dependent on H_2O_2 as addition of the enzyme catalase, which rapidly breaks down H_2O_2, blocks the inhibition. One could say, then, that an additional MOA of arginine may be its ability to potentiate the antagonistic effects of commensal streptococci.

More sophisticated weapons systems

We now know that many bacteria can communicate using diffusible small molecules. In gram-positive bacteria such as *S mutans*, these signals are usually small peptides ranging in size from as few as 7 amino acids to as large as 20 to 30 amino acids. One set of genes that is regulated by peptide signaling in *S mutans* is the production of bacteriocins, which are small antimicrobial peptides. Most strains of *S mutans* that have been examined have the capacity to produce a wide spectrum of antimicrobial molecules, often as many as 5 or 7 different compounds, that can kill or otherwise

inhibit related species. For example, the widely studied *S mutans* strain UA159 makes at least 5 different antimicrobial compounds. Some of these antimicrobial peptides are under the control of a peptide signaling system consisting of an 18-amino acid peptide called competence-stimulating peptide (CSP) and a specific signal transduction system that detects CSP and activates the genes for the antimicrobial peptide repertoire. Interestingly, commensal oral streptococci, such as *S gordonii* and the highly antagonistic *S* sp. A12, produce a protease (Sgc) that degrades CSP and blocks *S mutans*' ability to make certain factors that can kill commensals. *S* sp. A12, but not *S gordonii* or most other commensals, can also degrade a second critical peptide signal (*comX*-inducing peptide) and signal relay pathway (ComR) that are highly conserved in every *S mutans* isolate studied thus far.[27] Notably, arginine can exacerbate effects of signaling and diminish the ability of *S mutans* to sense and respond to its own peptide signals.

Summary and Future Directions

Clinicians, researchers, and lay persons have been observing dental plaque at a macroscopic and microscopic level for centuries. The use of mechanical and chemical strategies for the wholesale removal of oral biofilms has been a common practice for millennia. In the 1940s, the impact of fluoride on caries came to light, and fluoride's ability to strengthen enamel and to promote remineralization of damaged tooth tissue have made it the gold standard for prevention of caries, coupled with regular biofilm removal. Contemporary research on oral infectious diseases now embraces, and has provided additional support for, the ecological plaque hypothesis put forward by Marsh and others.[2] Slower to emerge have been technologies that can complement the action of fluoride by acting on oral biofilm ecology in ways that promote the stability of a health-associated flora—a flora with probiotic capabilities—and discourage the establishment, or reduce the cariogenicity, of aciduric pathogens. As described above, these advances have been hampered by an incomplete appreciation of the complexities of oral microbial ecology. In vitro and in vivo studies of the effects of arginine on caries and the microbiome demonstrate that arginine can be a highly effective anti-caries agent on its own or in combination with fluoride. Like many agents, for example, aspirin, recognition of the benefits has outpaced the development of a full understanding of all of the ways that the agents function. As microbiologists, biochemists, clinicians, and bioinformaticians unravel the intricacies of oral microbial ecology, we are likely to continue to discover new ways in which arginine can influence biofilm ecology and health. Given the combination of the clinical successes with arginine to date, the growing demand for noninvasive prevention and treatment approaches for caries, and the potential that arginine can impact other diseases (periodontal diseases), respiratory infections, and overall host responses by promoting the persistence of beneficial organisms in oral biofilms, research must continue apace to create a thorough understanding of what are likely many different mechanisms of action of arginine on human health.

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THE ROLE AND IMPACT OF ARGININE ON DENTAL CARIES THERAPEUTICS

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ABSTRACT

As a multifactorial disease, dental caries remains a global public health challenge despite extensive preventive measures, including worldwide use of fluoride toothpaste and mouthrinses, fluoridated water supplies, and application of sealants. Thus, novel and more effective approaches are needed to expand the arsenal to fight the problems associated with dental caries. About 40 years ago, researchers found that alkali generated by arginolytic bacteria through arginine catabolism can raise the pH of the oral biofilm microenvironment by neutralizing glycolytic acids, thus preventing an ecological microbial shift toward the acidogenic bacteria associated with tooth demineralization. Over the past several decades, increasing evidence from in vitro and in vivo studies has indicated that delivering exogenous arginine is a promising approach to prevent caries and even reverse the development of early lesions. The caries-preventive effect of arginine can be attributed to its ability to improve plaque pH stability, inhibit virulence factors of cariogenic pathogens, and modulate the oral microbiota toward a health-promoting microbial profile. For clinical applications, arginine can be incorporated into topical formulations, such as dentifrices, mouthrinses, and varnishes, and it functions much like a prebiotic. Furthermore, development of probiotics that can catabolize arginine and generate alkali is another potential direction for caries prevention. While several arginine-based oral care products are commercially available, their long-term anticaries efficacy needs to be validated in large-scale clinical studies.

Dental caries remains a global public health burden. According to the 2017 Global Burden of Disease report, the prevalence of untreated caries in primary and permanent teeth was 7.8% and 29.4%, respectively.[1] Although the caries prevalence rate has largely decreased in the past 50 years due, in large part, to the systemic and topical use of fluoride around the world,[2] caries remains one of the most common diseases globally because of inadequate prevention and treatment and an increase in sugar consumption. The key mechanism of fluoride in caries prevention is promoting remineralization on the tooth surfaces while having a minimal effect on the oral cariogenic microbiome. Hence, comprehensive measures targeting multiple caries risk factors are urgently needed to complement the anticaries effect of fluoride.

Novel strategies that have been proposed to further reduce caries prevalence and severity primarily include calcium- and phosphate-

based chemistries that increase tooth mineral saturation[3] and therapeutics to precisely target the oral microbiome.[4-6] With new insights into caries ecological etiology,[7] there is a growing appreciation of the crucial role of the eubiotic oral microbiome in maintaining oral health. Novel strategies for caries prevention that focus on microbial modulation rather than indiscriminate killing of oral bacteria are receiving increasing attention. These new strategies for combating dental caries include reengineering the microbiome by targeted suppression of specific cariogenic bacteria using vaccines,[8] bacteriophages,[4] or targeted antimicrobial peptides.[5] Other probiotic approaches include the displacement of *Streptococcus mutans* with engineered *S mutans* strains of reduced pathogenesis or with species that either compete with or directly antagonize *S mutans*.[6]

Despite the distinct conceptual strengths of each of the aforementioned approaches, they have not been developed into approved therapeutics to prevent dental caries yet. One agent, arginine, has recently received considerable attention for its potential to prevent caries by modulating the microbial community. Compelling in vitro and in vivo data are accumulating and indicate that arginine use leads to a healthy, noncariogenic microbiome by modifying the plaque microflora through promoting the growth of beneficial bacteria rather than having broad-spectrum bacterial killing. Thus, arginine displays great potential to develop into an effective therapeutic tool to complement fluoride applications for preventing dental caries.

The goal of this review is to provide researchers and clinicians with an update of the role and impact of arginine in caries therapeutics. We will elaborate on the mechanisms by which arginine improves homeostasis of the oral microbiome and summarize the applications and clinical outcomes of arginine-based technology for dental care.

Arginine and its role in oral health

As a semi-essential amino acid, arginine (L-arginine) is derived from exogenous dietary protein intake, endogenous body protein breakdown, and de novo arginine biosynthesis from citrulline.[9] Host-derived arginine can be secreted into the oral cavity via saliva as a free amino acid. Exogenous and endogenous arginine-containing peptides/proteins can be broken down by proteases produced by oral microbiota to release free arginine. The concentration of free arginine in stimulated, parotid ductal saliva of healthy adults is about 14.61 nM/mL.[10]

In the late 1970s and 1980s, Kleinberg's group reported that alkali generated by arginolytic bacteria played a major role in maintaining oral microenvironmental pH, especially in the presence of fermentable carbohydrates in in vitro studies.[11-13] In a subsequent clinical study, Van Wuyckhuyse and colleagues[10] showed that caries-free adults had significantly higher free arginine levels in stimulated, parotid ductal saliva than individuals with a history of dental decay. The concentration of arginine within dental plaque formed 48 hours after oral hygiene procedures was measured and determined to be approximately 0.22 mM/mg plaque,[14] indicating that arginine can be taken up by oral biofilms. Furthermore, the amount of generated ammonia (NH_3), a product from arginine catabolism, was higher in plaque samples than in saliva samples from the same individuals.[15] Because caries is a biofilm-dependent oral disease, uptake and active metabolism of arginine in dental plaque suggests its potential impact on the plaque microbiome, implying a close relationship between arginine and oral health. These early-stage laboratory and clinical findings opened a new research field in oral health that spans basic, translational, and clinical studies pertaining to the role and impact of arginine in caries therapeutics.

Metabolism of arginine by oral bacteria

Arginine can be metabolized by bacteria through 2 pathways: the arginine deiminase system (ADS) and the agmatine deiminase system (AgDS). Studies reported that ADS activity is higher in the plaque and saliva of caries-free individuals than in caries-active children[16] and adults.[15] Expression of ADS can be induced by arginine and low pH,[17] which plays an important role in pH homeostasis via microbial modulation. AgDS has been associated with acid resistance in bacteria. Its activity in oral biofilms is generally lower than that of ADS.[18]

The arc operon of ADS is composed of 4 genes: *arcA, arcB, arcC*, and *arcD/arcE*. Arginine-ornithine antiporter, a membrane-embedded protein encoded by *arcD/arcE*, is involved in transporting arginine from the extracellular environment into cytoplasm. Intracellular arginine can be catabolized by arginine deiminase, an enzyme encoded by *arcA*, into citrulline and ammonia. Citrulline can be further broken down by *arcB*-encoded ornithine carbamoyl transferase into ornithine and carbamoyl phosphate; the former is then transported out of the cell by arginine-ornithine antiporter through electroneutral ornithine/arginine exchange. Carbamoyl phosphate is further catabolized by *arcC*-encoded carbamate kinase into ammonia and carbon dioxide (CO_2), concomitantly donating the phosphate to adenosine diphosphate and producing adenosine triphosphate[19] (Figure 1). Genomic and phenotypic analyses revealed the presence of ADS in multiple bacteria species, most of which are commensal, including *Streptococcus sanguinis, Streptococcus gordonii, Streptococcus mitis, Streptococcus oralis, Streptococcus rattus, Streptococcus parasanguinis*, and *Streptococcus cristatus*; certain *Lactobacillus* and *Actinomyces* species; and a few spirochetes.[7,20]

Arginine in dental biofilms also can be catabolized by the bacterial enzyme arginine decarboxylase into agmatine, which can be further broken down via AgDS. The key genes that encode AgDS-related functions, *aguA, aguB, aguC*, and *aguD*, are often organized into one operon.[17,21] *AguD* encodes an agmatine-putrescine antiporter, which enables the entry of free agmatine into the cell and transport of putrescine out of the cell. Agmatine can be hydrolyzed to N-carbamoylputrescine and ammonia by agmatine deiminase encoded by *aguA*. The putrescine carbamoyl transferase encoded by *aguB* further metabolizes N-carbamoylputrescine to generate putrescine and carbamoyl phosphate. Carbamate kinase, encoded by the *aguC* gene, transfers a phosphate group from carbamoyl phosphate to adenosine diphosphate to generate adenosine triphosphate, CO_2, and ammonia. AgDS is present in multiple oral bacteria, including *S mutans, Streptococcus sobrinus, Streptococcus downeii, S rattus, Streptococcus uberis, S mitis, Streptococcus cricetus, S sanguinis*, and *Streptococcus salivarius*, as well as *Lactobacillus salivarius* and *Lactobacillus brevis*.[17] Researchers have recognized that arginine metabolism using AgDS cannot generate enough base to increase the microenvironment pH of oral biofilms; rather, its main function is to enhance bacterial acid tolerance by increasing cytoplasmic pH through ammonia and adenosine triphosphate generation.[18]

Metabolism of arginine improves pH homeostatis

Although caries is a multifactorial disease involving the interactions between host, bacteria, diet, and environmental factors,

Figure 1

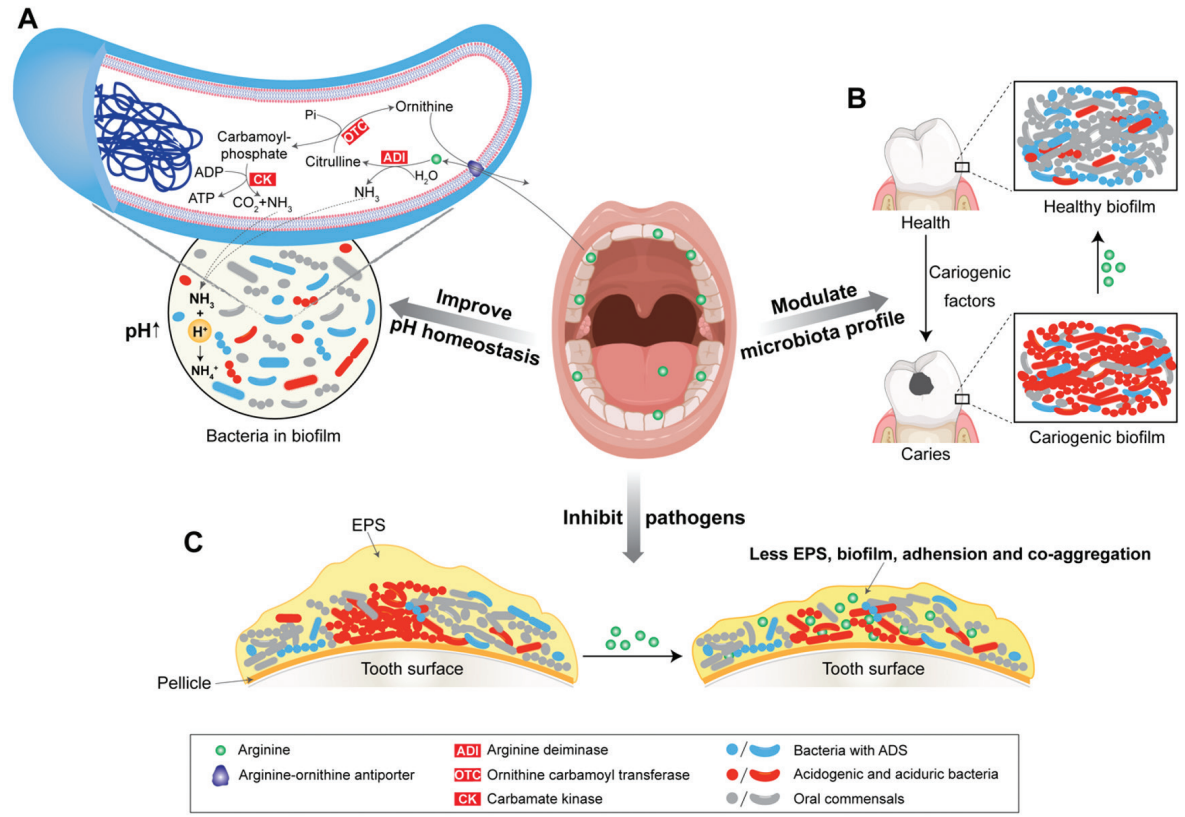

Figure 1. *The role of arginine in caries therapeutics.* **A.** *Arginine can be transported into arginine deiminase system positive (ADS^+) bacteria via arginine-ornithine antiporter and hydrolyzed by enzymes (ADI, OTC, and CK) in ADS. Metabolic products from ADS include ornithine, NH_3, carbon dioxide (CO_2), and adenosine triphosphate (ATP). Ammonia (NH_3) picks up an H+ from the acid to produce ammonium ion (NH_4^+) and results in a rise in cytoplasmic and environmental pH.* **B.** *Alkali generated from arginine metabolism could modulate the microbiota profile by creating an alkaline microenvironment, facilitating the growth of oral commensals and ADS^+ bacteria, while inhibiting the growth of acidogenic and acid-tolerant bacteria.* **C.** *Arginine can be taken up by dental biofilms and may directly inhibit extracellular polymeric substance (EPS) production, biofilm formation, adhesion, and co-aggregation of certain pathogens, especially acidogenic, cariogenic bacteria.*

the key determinant of caries is the pH decrease on the tooth surface due to bacterial fermentation–related acid production. As illustrated in the pH curves from Stephan and Miller[22] in 1943, when dental plaque is exposed to fermentable carbohydrates, the pH falls rapidly, then gradually returns to starting levels. Factors such as salivary washing and alkali generation are of great importance for the pH-raising phase. Ammonia molecules produced via the ADS are protonated by the acid to produce ammonium ion ($NH4^+$), resulting in an increase in cytoplasmic and environmental pH.[23] When the pH is lower than 7.0, more than 99.4% of the ammonia molecules are protonated to ammonium.[24] Neutralization of glycolytic acids by ammonia helps maintain a favorable remineralization–demineralization equilibrium on the tooth surface. Clinical observations revealed that caries-free individuals have higher concentrations of ammonia and higher resting pH values in their dental plaque compared with caries-susceptible individuals.[25]

Using an in vitro multispecies biofilm model established by our group, we simulated the in vivo arginine uptake and catabolism process in dental plaque. This human saliva–derived in vitro multispecies microbial community was recovered using SHI medium,[26] and the cultured community contained more than 100 species of oral bacteria, thus approaching the diversity and overall metabolic functionality of the human oral microbiome.[27,28] Most importantly, this in vitro system allows the accurate simulation of the pH drop and recovery in dental plaque after a glucose challenge,[29] as observed in vivo. From this study, we found that treatment of in vitro biofilm with only sucrose led to a sustained pH drop from 7 to 4.5, while biofilms preincubated with arginine displayed a significant capacity to recover to a higher pH after a sucrose challenge. Our data suggests there is active uptake and metabolism of arginine within the dental biofilm, which would contribute to improved pH homeostasis, especially under acid stress.[30] Consistent with our findings, Ledder and colleagues[31] also demonstrated that sustained 1.5% (weight/volume) arginine treatment of an in vitro plaque microcosm significantly increased plaque pH with or without 5% sucrose supplementation.

Metabolism of arginine modulates biofilm microbial profile

Findings from multiple in vitro studies have suggested that the presence of arginine may alter the biofilm species composition (Figure 1). In our in vitro biofilm model, a shift in the community structure and increased species diversity were observed in the group treated with 75 mM arginine compared to the untreated group. Furthermore, the microbial community's capability to recover from a lowered pH after a sucrose challenge was significantly enhanced, demonstrating that the presence of arginine led to improved pH homeostasis through changes in the microbial community.[30] By using *S mutans, S sobrinus*, and the ADS-positive (ADS^+) species *S sanguinis* and *S gordonii*, Berto and colleagues[32] constructed an in vitro cariogenic biofilm on enamel surfaces The authors reported that treatment with arginine-containing sodium mono-fluorophosphate toothpaste resulted in a higher proportion of ADS^+ bacterial species within the biofilm, higher culture media pH, and lower loss of enamel hardness when compared with use of the sodium mono-fluorophosphate toothpaste without arginine. In another in vitro study[33] using saliva-derived biofilms, investigators reported that treatment with a combination of 2.5% arginine and 500 ppm NaF resulted in the least demineralization on the enamel surface, followed by 500 ppm NaF alone, 2.5% arginine alone, and the

phosphate-buffered saline control. In addition, 2.5% arginine alone reduced the *S mutans* to *S sanguinis* ratio by inhibiting *S mutans* within biofilms, whereas 500 ppm NaF failed to affect the ratio because of its inhibitory effect on both *S mutans and S sanguinis*. This result was corroborated by other studies,[30,32] supporting the ability of arginine to modulate microbial structure.

Metabolism of arginine by arginolytic bacteria contributes to an alkaline environment in dental plaque ecology, creating less favorable conditions for acidogenic and aciduric bacteria to thrive. According to the currently accepted ecological plaque hypothesis,[7] bacterial metabolic acid production promotes a microenvironment favoring the growth of acidogenic and aciduric bacteria. This shift leads to excessive acid production, resulting in a more cariogenic microbiome, which drives tooth demineralization and the development of caries. Thus, alkali generated from bacterial arginine metabolism via ADS or AgDS is likely to be able to enhance the microbial ecological stability in the dental plaque by modulating the microflora profile, particularly in the presence of fermentable carbohydrates. The contribution of arginine metabolism to pH homeostasis via microbial community modulation makes it a potential candidate for effective caries prevention.

Arginine inhibits biofilm formation, adhesion, and co-aggregation of certain pathogens

In addition to the metabolism-related pH raising and microflora modulation capability, the presence of arginine itself may directly inhibit certain pathogens in dental biofilms, especially those recognized as cariogenic (Figure 1). Notably, arginine mainly affects the cariogenicity of *S mutans* by inhibiting its virulence factors rather than by directly suppressing bacterial growth.[34] Studies focusing on *S mutans* reported that arginine could inhibit *S mutans* biofilm formation, adhesion, and co-aggregation, thus reducing its pathogenic potential.[34-36] The mechanism underlying this biological property is the arginine-induced downregulation of genes involved in virulence, such as attachment/accumulation (*gtfB* and *spaP*), bacteriocins (*nlmA, nlmB, nlmD*, and *cipB*), and the sigma factor required for competence development (*comX*).[34] The downregulation of these genes affects water-insoluble extracellular polymeric substance (EPS) denseness[35,36] and other pathogenic abilities,[34] leading to attenuated virulence. In vitro studies using multispecies biofilms with *S mutans*[36,37] demonstrated that arginine significantly reduced the amounts of insoluble EPS and the biomass of the polymicrobial biofilms, and disrupted the dynamic microbial interactions associated with pathogenic biofilm development.

The development of dental biofilms is a well-regulated process. *Fusobacterium nucleatum*, considered a bridging organism, was found to play a crucial role in linking early and late colonizing bacteria during multispecies biofilm maturation.[38] Kaplan and colleagues[38] showed that arginine can inhibit RadD, an *F nucleatum*–encoded arginine-inhibitable adhesin that mediates co-aggregation between *F nucleatum* and early colonizing gram-positive species. Arginine was also found to inhibit co-aggregation between *Porphyromonas gingivalis* and mutans streptococci.[39] These results suggest that arginine could interrupt cell-to-cell co-aggregation in plaque biofilms and, in doing so, reduce biofilm biomass as well as inhibit synergistic pathogenesis potentially derived from direct physical interaction among different pathogenic bacteria.

Application of arginine in caries therapeutics

Applications of arginine in caries therapeutics, particularly in caries prevention, include incorporating arginine into oral care formulations, using arginine as a prebiotic, and developing ADS^+ bacteria as potential probiotics. Many arginine-based oral products have already been marketed. So far, the major goal of arginine-based products is caries prevention or treatment. Therefore, this review summarized applications and clinical outcomes of arginine-based technology in oral care, aiming to provide researchers and clinicians with the current understanding of the role and impact of arginine on caries therapeutics.

Arginine-based oral care products

The most frequently used and well-studied arginine-based products in oral care are arginine-containing dentifrices with a calcium-based abrasive system, in which arginine is either the sole effective agent or in combination with the anticaries agent fluoride. Other applications of arginine in oral care products include mouthrinses[40,41] and varnishes.[42] These products have been found to achieve similar anticaries effects as arginine-containing dentifrices.[40-42]

An in vivo study comparing 1.5% arginine, fluoride-free toothpaste with fluoride-containing toothpaste (1100 ppm fluoride as NaF) in 19 caries-free and 19 caries-active individuals twice daily for 4 weeks[43] showed that arginine significantly increased ADS activity in plaque samples from the caries-active individuals. The plaque microbial profiles of caries-active participants treated with arginine displayed a shift in bacterial composition closer to the composition of caries-free participants. In another in vivo study using the same toothpaste in 83 adults, Nascimento and colleagues[44] found that arginine significantly increased ADS activity and pH values of dental plaque after incubation with glucose, which was corroborated by their previous study.[43] Clinically, 14% of active caries lesions became inactive after 12 weeks, although no difference was observed between arginine or fluoride treatments[44]. Study findings also indicated that the anticaries mechanisms of arginine and fluoride are different; while arginine metabolism promotes biofilm pH homeostasis, fluoride enhances resistance of tooth minerals to low pH, and reduces acid production in supragingival oral biofilms.[44]

Arginine and fluoride have anticaries therapeutic effects, albeit with different mechanisms. Researchers have proposed that the therapies complement each other, resulting in improved protection from caries. Arginine's ability to increase microenvironmental pH and reduce biofilms' EPS matrix may allow fluoride to be more effective in promoting tooth remineralization and allowing antimicrobials to penetrate biofilms.[45] An increasing number of in vivo studies have investigated the anticaries efficacy of arginine when combined with other agents, such as fluoride and calcium, and compared its efficacy with that of fluoride only. A 2-year pivotal clinical study involving 6,000 participants showed that dentifrices containing 1.5% arginine, an insoluble calcium compound, and 1450 ppm fluoride provided significantly greater protection against caries lesion, in a low to moderate caries risk population, than dentifrices containing 1450 ppm fluoride alone.[46]

Studies using the same dentifrice formulations in 331 children[47] and 3779 adults[48] revealed that dentifrices with arginine exhibited superior efficacy in arresting and reversing active coronal caries lesions in children and active root caries lesions in adults. Meanwhile, testing of other similar dentifrices containing arginine as the effective agent, such as an arginine bicarbonate/calcium carbonate (CaviStat)–containing dentifrice, demonstrated more effective inhibition against caries initiation and progression compared with fluoride toothpaste controls.[49,50] Results from meta-analyses indicated that dentifrices containing 5000 ppm fluoride or 1.5% arginine plus

1450 ppm fluoride were more effective in inactivating root caries lesions than dentifrices containing 1100 to 1450 ppm fluoride,[51] suggesting that arginine could enhance the efficacy of fluoride while reducing potential side effects of high concentrations of fluoride, including fluoride toxicity and dental fluorosis.[52]

In in vivo anticaries dentifrice studies, the most frequently used concentration of arginine was 1.5%, which achieved similar anticaries efficacy as that in studies using 8% arginine.[33,53] Additionally, in a clinical study,[54] use of toothpaste containing 8% arginine, calcium carbonate, and 1450 ppm fluoride significantly reduced tooth sensitivity compared to a leading toothpaste containing 2% potassium ions. Mechanisms behind tooth hypersensitivity relief include physical sealing of dentin tubules with plugs that contain arginine, calcium carbonate, and phosphate.[54] These plugs resist normal pulpal pressures and acid challenge and can effectively reduce the change in dentin fluid flow, which is the primary cause of hypersensitivity.

Arginine functions like a prebiotic

Prebiotics are defined as substrates that are selectively used by host microorganisms to confer a health benefit.[55] L-arginine has been used as a dietary supplement to improve human health conditions such as cardiovascular diseases. Arginine is a precursor of nitric oxide, which plays important roles in vasodilatation, bacterial challenge and cytokine stimulation, and platelet aggregation.[56] In a 1-year study,[57] use of a sugarless tablet containing CaviStat (an arginine bicarbonate calcium carbonate complex) led to inhibition of caries onset and progression. Thus, arginine may also serve as a promising prebiotic in oral care.

Despite most in vivo clinical studies showing effective anticaries outcomes with no potential risks, there are 2 concerns related to using arginine in oral care products[58]: (1) Many studies were sponsored by companies manufacturing the tested products, which has the potential to increase the bias, although this is not uncommon in pharmaceutical trials and is an inherent and necessary part of therapeutic development; (2) Some studies raised potential concerns about arginine-related adverse effects, including discomfort, oral hygiene deterioration, tooth staining, increased periodontal disease, and oral malodor.[58,59] Hence, the long-term efficacy and safety of these arginine-based clinical products require further investigation in high-quality, well-designed, non–industry-supported clinical studies.

Probiotic potential of ADS$^+$ bacteria

A probiotic is defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host."[60] Use of probiotics in improving gastrointestinal health has a long history and is highly commercialized.[60] More recently, attention has turned to the role of probiotics in oral health, particularly in caries, periodontitis, and oral candidiasis therapeutics.[6] The presence of ADS varies between different species, particularly in *Streptococcus* and *Actinomyces* species, as well as between different strains of individual species.[61-63] Probiotics containing ADS$^+$ bacteria, intending to increase arginine catabolism activity within dental plaque, could be effective in preventing or even reversing the cariogenic effect of biofilms. Some studies[63,64] investigated the potential of arginolytic, commensal streptococci as probiotics that could have a positive impact on the oral microbiome by increasing the local pH while antagonizing cariogenic pathogens. However, candidate strains need to be carefully selected and extensively tested before being considered for clinical applications.

CONCLUSION

Arginine is a promising anticaries therapeutic with well-documented caries prevention ability. Its anticaries properties can be attributed to the following key mechanisms: (1) arginine catabolism by arginolytic bacteria, which improves pH stability and modulates the biofilm microbiota through ammonia production; and (2) arginine-mediated inhibition of adhesion and co-aggregation and reduction of certain cariogenic bacteria in biofilms. Arginine-based oral care formulations, arginine as a prebiotic, and probiotics including ADS$^+$ commensal bacteria are 3 potential applications that can bring arginine-based approaches into caries therapeutics. To date, arginine-based oral health products have demonstrated satisfactory in vivo efficacy in preventing and even reversing early enamel carious lesions.

Future exploration of arginine applications in caries prevention should focus on the following: (1) combining arginine therapies with existing fluoride therapies to achieve better anticaries efficacy; (2) developing arginine-based products as alternative anticaries options for individuals seeking fluoride-free products and children at risk of dental fluorosis; (3) exploring the application of arginine as a prebiotic in tandem with ADS$^+$ bacterial strains as probiotics in the oral cavity to achieve long-term microflora ecological homeostasis through the ability of arginine to promote the growth of ADS$^+$ bacterial species; and (4) combined application of arginine with targeted antimicrobial treatments to suppress cariogenic bacteria in a long-lasting, stable, anticaries effect because arginine can help maintain a healthy biofilm microenvironment after elimination of cariogenic bacteria. Although the potential of arginine developing into an anticaries therapeutic is clearly demonstrated by in vitro and in vivo data, more clinical studies are needed to evaluate long-term anticaries efficacy and potential adverse effects before commercializing arginine-based products.

Disclosure

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CLINICAL PERSPECTIVE: ARGININE AS AN ANTICARIES AGENT

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ABSTRACT

Globally, an estimated 2 billion people experience caries of permanent teeth and 514 million children suffer from caries of primary teeth. The use of fluoride as a caries preventive agent continues to be the primary preventive strategy deployed in the fight against caries worldwide. However, based on the persistent high caries rates and for some populations, especially in areas of the world where endemic fluorosis exists, it is prudent to continue to explore the potential for new actives and strategies for caries prevention. The most widely studied alternative preventive strategy which has been identified utilizes the naturally occurring amino acid, arginine, as a prebiotic to enhance the mouth's natural defense mechanism against caries by helping to maintain plaque pH in a neutral state while maintaining or fostering the return to a healthy microbiome. As the use of non-fluoride toothpastes, with no known actives to address caries, continues to grow, arginine may be a choice that is equally as effective at preventing caries albeit by a different mechanism than fluoride. The complementary mechanisms of action of fluoride to strengthen the enamel and arginine to restore the microflora and oral environment to a healthier state have been shown to be a winning combination demonstrating superior efficacy and the best caries prevention results reported in the literature thus far.

Introduction

For over 60 years, since the advent of the first mass-marketed toothpaste in 1955, the majority of caries prevention strategies have been centered around the use of fluoride. This strategy focuses on making the tooth more resistant to acid damage by allowing for the replacement of the hydroxyl ions in hydroxyapatite with fluoride-forming fluorapatite, resulting in increased hardness of tooth mineral. The topical application of fluoride also enhances tooth remineralization. The use of fluoride is not unwarranted; clinical studies support significant reductions (23%) in caries incidence with fluoride use,[1] and the FDA recognizes several fluoride sources as generally safe and effective for the prevention of caries. However, even with the use of fluoride, caries as a disease remains prevalent today, affecting 60% to 90% of all schoolchildren and the vast majority of adults.[2] Furthering this point, the World Health Organization Global Oral Health Status Report (2022) estimated that oral diseases affect close to 3.5 billion people worldwide, with 3 out of 4 people affected living in middle-income countries.[3] Globally, an estimated 2 billion people suffer from caries of permanent teeth and 514 million children suffer from caries of primary teeth. This number is staggering and makes caries twice as common as headaches.[4]

Based on these early findings, the use of fluoride as a caries preventive agent continues to be the primary preventive strategy deployed in the fight against caries worldwide. However, based on the persistent high caries rates and for some populations, especially in areas of the world where endemic fluorosis exists, it is prudent to continue to explore the potential for new actives and strategies for caries prevention. Furthermore, in addition to areas of the world where endemic fluorosis is a concern, there has also been a growing consumer segment that actively seeks alternatives to fluoride products to sustain their oral health. These consumers have become concerned with multiple sources of fluoride ingestion and

the potential health outcomes that could arise with exposure to excessive fluoride. Regardless of whether these concerns are justified, they have become an important consideration for consumers when choosing over-the-counter caries prevention products for themselves and their families. An increasing number of consumers choose to brush with fluoride-free alternative toothpastes, believing that these products present a viable alternative for caries prevention while addressing their perceived concerns about the safety of fluoride.[5] A recent evidence-based review supports the use of fluoride-free toothpastes as cleaning agents to remove debris in the oral cavity and to potentially reduce gingivitis while emphasizing the fact that toothpastes without a caries preventive active such as fluoride have failed to show a benefit in terms of reducing the incidence of dental caries.[6] To address these aforementioned concerns and findings, an effective alternative to fluoride has been explored and studied alone and in combination with fluoride, as it is clear that fluoride alone has been unable to address the significant burden of caries worldwide.

The most widely studied alternative preventive strategy that has been identified utilizes the naturally occurring amino acid, arginine, as a prebiotic to enhance the mouth's natural defense mechanism against caries by helping to maintain plaque pH in a neutral state while maintaining or fostering the return to a healthy microbiome. Unlike fluoride, which has a chemical mode of action that targets the tooth, arginine is metabolized by arginolytic bacteria through the Arginine Deiminase System (ADS), which produces ammonia, helping to modulate the pH and resulting in a more neutral environment, shifting the dysbiotic microbiome to one that is healthier and less caries-prone.[7,8] To date, numerous clinical studies have been conducted with toothpastes containing arginine at various levels (typically 1.5% to 8%). While arginine with calcium carbonate was at first most widely used in dentistry to occlude dentin tubules to prevent sensitivity, arginine is now proving to be a new and useful active in controlling caries. Arginine is a natural, safe amino acid found in saliva as well as in food products, dietary supplements, baby food, formula, and breast milk. It is not surprising that arginine is found at higher concentrations in the saliva of people who are caries free versus the low levels detected in people with a history of dental decay.[9,10] It appears to be our body's own protective measure against caries. This review will focus on results from key clinical trials that have explored the use of arginine (in the presence of a calcium source) as an anticaries agent (with and without fluoride), laying the foundation for future studies to support this new caries preventive agent as an alternative and/or complementary choice to be considered in our fight against caries.

Arginine with Fluoride

Research on arginine combined with fluoride has been conducted based on years of *in vitro* and *in vivo* research. The combination of arginine to address pathogenic microbial shifts with fluoride known to strengthen the enamel and to make it more resistant to decay addresses 2 processes across the caries continuum, which clinically has been proven to be more effective than focusing on a single process. In this combined strategy, arginine acts through targeting bacteria that are implicated in caries development, while fluoride helps by strengthening enamel and remineralizing surfaces of the teeth that had become compromised.

Years of research on arginine combined with fluoride has given support for dual use. The body of work for arginine plus fluoride, while not as extensive as fluoride alone, is by no means small; in total, over 25 clinical studies have been conducted on this technology (Table1). Clinical research in this area of arginine with fluoride has been conducted over a decade and has included over 18,000 subjects. Clinical studies on arginine and fluoride can be classified into 4 types: plaque metabolism, microbiome, *in situ* intraoral, and caries assessments. The results from these studies, taken in aggregate, support the use of arginine and fluoride to achieve an additive, if not synergistic, effect on caries prevention far exceeding previous clinical results seen with fluoride alone. In addition, these studies clearly demonstrate the ability of arginine, a newly identified active against caries, to make substantial contributions to caries prevention strategies.

A summary table of key arginine plus fluoride clinical studies is included in Table 1.

Plaque Metabolism Studies

As previously mentioned, throughout the history of caries prevention, the bulk of applications have been centered around fluoride and its ability to harden weakened enamel. However, there has been a movement to address the microbes and acidic environment that initiates the caries process, resulting in caries prevention strategies that focus on the source of caries, pathogenic plaque bacteria. Arginine has been studied to determine if it can produce a healthier microbial environment by limiting acidogenic bacteria while favoring arginolytic base–producing bacteria.[7,11,12] In Wolff's study, participants in the test group brushed for 2 weeks with

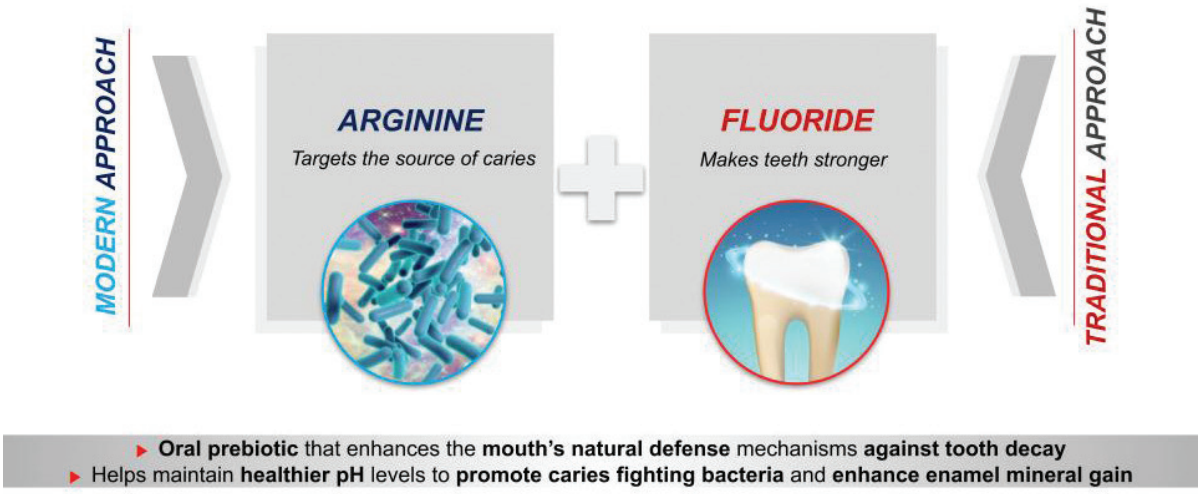


Figure 1. *Benefits of adding a modern approach to traditional approaches for caries management*

Table 1. *Key Arginine Plus Fluoride Clinical Studies*

Author	Year	Population	Test Article(s)	Control	Endpoint	Results (improvements above and beyond fluoride controls)
Caries Clinical Studies						
Kraivaphan *et al.*	2013	Children	1.5% Arg (DiCal) + 1450 ppm F; 1.5% Arg(Calcium Carbonate) + 1450 ppm F	1450 ppm F	DMFT Scores @ 2 years	% Change (reduction in new cavities) = 17.7 - 21%
Li *et al.*	2015	Children	1.5% Arg (DiCal) + 1450 ppm F; 1.5% Arg (Calcium Carbonate) + 1450 ppm F	1450 ppm F	DMFT Scores @ 2 years	% Change (reduction in new cavities) = 20.5%
Petersen *et al.*	2015	Children	1.5% Arg + 1450 ppm F	1000 ppm F	DMFT Scores @ 2 years	% Change (reduction in new cavities) = 34 - 41%
Early Caries Clinical Studies						
Yin *et al.*	2013a	Children	1.5% Arg + 1450 ppm F	1450 ppm F; 0 ppm F	QLF measuring ΔQ values @ 6 mos	Arginine + F vs non fluoride showed 44.3% improvement in early caries
Yin *et al.*	2013b	Children	1.5% Arg + 1450 ppm F	1450 ppm F; 0 ppm F	QLF measuring ΔQ values @ 6 mos	Arginine + F vs non fluoride showed 43.2% improvement in early caries
Srisilapanan *et al.*	2013	Children	1.5% Arg + 1450 ppm F	1450 ppm F	QLF measuring ΔQ values @ 6 mos	Arginine + F showed 44.6% improvement from baseline
Arginine *in situ* Studies						
Cantore *et al.*	2013	Human Enamel	1.5% Arg (DiCal) + 1450 ppm F; 1.5% Arg (Calcium Carbonate) + 1450 ppm F	250 ppm F (Negative Control); 1450 ppm F (Positive Control)	Remineralization	4x greater remineralization in test groups than fluoride alone
		Bovine Enamel	1.5% Arg (DiCal) + 1450 ppm F	250 ppm F (Negative Control); 1450 ppm F (Positive Control)	Demineralization	Prevented demineralization and showed remineralization
		Bovine Enamel	1.5% Arg (CC) + 1000 ppm F	0 ppm F (Negative Control); 1000 ppm F (Positive Control)	Demineralization	4x less demineralization in test group than fluoride alone
Plaque Metabolism Studies						
Cantore *et al.*	2013	Adults (18-70 y.o)	1.5% Arg + 1000 ppm F	0 ppm F (Negative Control); 1000 ppm F (Positive Control)	Ammonia Production @ 2 weeks	1.8x directionally higher (statistically significant) ammonia production was observed
		Adults (18-65 y.o)	1.5% Arg + 1000 ppm F	0 ppm F (Negative Control); 1000 ppm F (Positive Control)	Lactate Production @ 2 weeks	1.2x directionally lower (but not statistically significant) lactate production was observed
Santarpia *et al.*	2014	Adults	1.5% Arg + 1450 ppm F	1450 ppm F	Lactate Production @ 12 weeks	No significant difference between test and control
					Ammonia Production @ 12 weeks	No significant difference between test and control
					pH @ 12 weeks	Resting and terminal pH of the test group was statistically significantly higher than the control group
Wolff *et al.*	2010	Adults	1.5% Arg + 1450 ppm F	1450 ppm F	Lactate Production @ 2 weeks	Groups were not statistically significant
					Ammonia Production @ 2 weeks	The difference between the test and control groups during the treatment period was statistically significant
					pH @ 2 weeks	The resting pH of the test group was statistically significantly higher than the resting pH of the control group. pH 2 weeks post treatment was not statistically significant.
Microbiome Studies						
Carda-Diéguez *et al.*	2022	Adults	1.5% Arg + 1450 ppm F	1450 ppm F	Metagenomic and metatranscriptomic analyses of human dental plaque	Fluoride or F + Arg-containing dentifrice had an overall positive effect on the composition and functional profle of the dental plaque microbiota. This observation was especially pronounced for the F + Arg dentifrice.

a dentifrice containing 1.5% arginine and 1450 ppm fluoride.[11] This arginine and fluoride toothpaste was shown to significantly increase plaque's ability to convert arginine to ammonia relative to brushing with a control dentifrice containing only 1450 ppm fluoride. The resting pH, which is plaque's natural pH in the absence of a sucrose challenge, of both the arginine plus fluoride and the control fluoride groups was also examined. Typically, resting pH ranges from 6.8 to 7. Under resting pH conditions, plaque is supersaturated with respect to enamel and there is a positive driving force favoring remineralization. An increase in resting pH increases the degree of saturation of the plaque fluid with respect to enamel and increases the force behind enamel remineralization. It was observed that the resting pH of the group who used the arginine- and fluoride-containing toothpaste was significantly higher than the group using the control fluoride product at the end of the 2-week treatment period ($P \leq 0.01$; 7.31 for the arginine/fluoride group versus 7.1 for the fluoride group). This result signals that enamel remineralization would be favored in the arginine and fluoride group over the fluoride alone control group.

In Situ Intra-Oral Studies

Another way of assessing the ability of dentifrices to remineralize tooth structure and to withstand demineralization *in vivo* is to observe their performance during *in situ* intraoral studies. These studies are typically designed to assess either remineralization–demineralization (remin-demin) or demineralization–remineralization (demin-remin). Remin-demin studies measure mineral gain to a surface, whereas demin-remin studies examine mineral loss or demineralization. A remin-demin clinical model was used to assess the ability to promote remineralization of enamel by 2 dentifrices containing different formulations of 1.5% arginine and 1450 ppm fluoride, as sodium monofluorophosphate (MFP), relative to a positive control with dicalcium phosphate dihydrate (Dical) and 1450 ppm fluoride and a negative control with Dical and 250 ppm fluoride.[12] Microradiography and image analysis were used to measure mineral changes. The study used a double-blind crossover design with a 2-week treatment period. Products were used twice a day for 2 weeks. The percent mineral changes were +18.64, +16.77, +4.08, and −24.95 for the 1.5% arginine/Dical/1450 ppm fluoride, the 1.5% arginine/calcium carbonate/1450 ppm fluoride, the positive control with 1450 ppm fluoride alone, and negative control with 250 ppm fluoride alone, respectively. The positive control was statistically significantly better than the negative control in promoting remineralization ($P = 0.0001$), which indicates the study results are valid. The 2 arginine-containing test products showed statistically significant benefits beyond the positive control ($P < 0.05$) (Figure 2). No significant difference was observed in efficacy between the 2 arginine- plus fluoride-containing products, indicating that efficacy in promoting remineralization was independent of the choice of dical or calcium carbonate as the source of insoluble calcium in the formula.

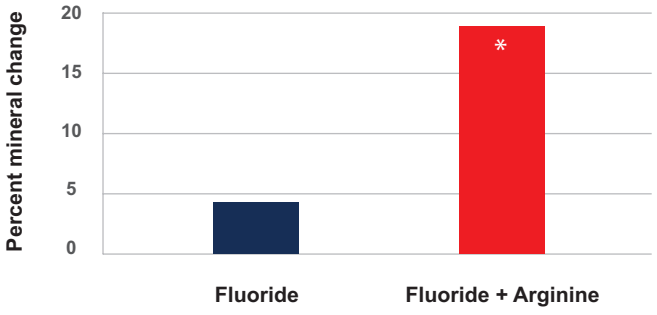
In a demin-remin study, a dentifrice containing 1.5% arginine, dicalcium phosphate dihydrate, and 1450 ppm fluoride was compared to matching positive and negative controls with 1450 ppm and 250 ppm fluoride, respectively, as was described in Cantore.[12] Net mineral loss was experienced for both positive and negative controls. The observation that the positive control was statistically significantly more effective in preventing mineral loss than the negative control validates the benefits of the arginine technology. The percent demineralization values were −8.50, +1.67, and +12.64 for the 1.5% arginine/Dical/1450 ppm fluoride, the positive control, and negative control dentifrices, respectively. No net mineral loss was experienced following the use of the arginine- plus fluoride-containing dentifrice; enamel specimens actually showed an increase in hardness after the treatment period. Importantly, the arginine- plus fluoride-containing dentifrice was shown to be statistically significantly more effective than the matched positive control dentifrice in preventing demineralization of enamel. These results indicate that arginine is playing a significant role in enhancing the efficacy of fluoride.

Caries Studies

There are a number of studies with fluoride and 1.5% arginine that look closely into how these ingredients may impact the caries continuum. Early caries lesions were assessed using the quantitative light-induced fluorescence method.[13-15] Arginine and fluoride, when formulated in a dentifrice, showed 2 times the reversal of early caries lesions as compared to 1450 ppm fluoride alone (Figure 3).

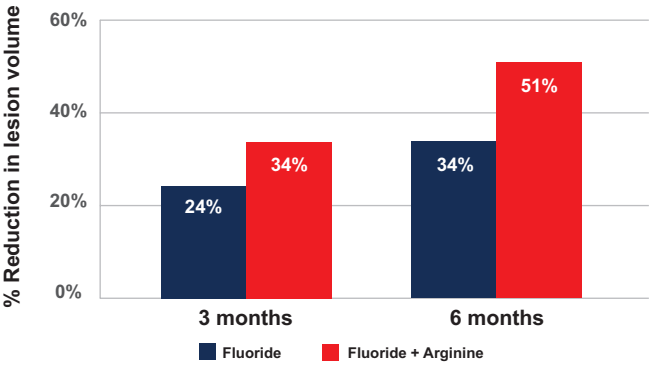
Longer studies on fluoride and arginine dentifrices have also been conducted, all demonstrating the improved efficacy of arginine and fluoride toothpastes against caries.[16] In one 2-year study, a double-blind, randomized, unsupervised, parallel-group clinical trial on over 5,500 children in China on the efficacy of arginine and fluoride was demonstrated.[17] In this study, the 2 test dentifrices contained 1.5% arginine, 1450 ppm MFP, and an insoluble calcium compound, whereas the positive control dentifrice contained 1450 ppm fluoride in a silica base. The children were examined at baseline as well as 1 and 2 years after continuous product usage. After 1 year of use, there were no statistically significant differences among the 3 groups with respect to decayed, missing, and filled teeth (DMFT) or to decayed,

Figure 2. *Arginine significantly enhances remineralization as shown in Cantore 2013*



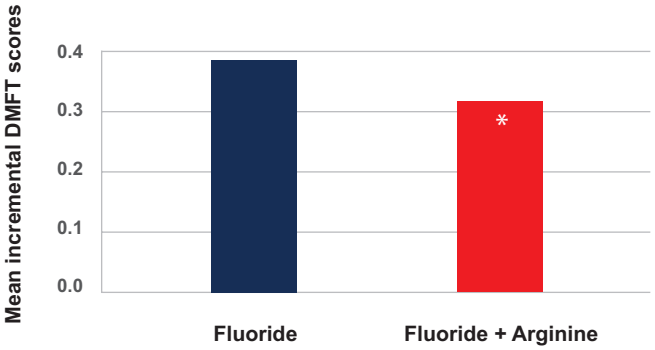
**Statistically significant versus fluoride control, p<0.05*

Figure 3. *Reduction in lesion volume for Fluoride and Fluoride + Arginine at 3 months and 6 months as shown in Yin 2013 b. 34% reduction in caries volume was seen at 3 months for the arginine and fluoride combination dentifrice compared to a 34% reduction seen after 6 months of fluoride alone dentifrice use. Comparatively, at 6 months, the arginine and fluoride dentifrice had a 51% reduction in lesion volume demonstrating the additive effect of combining arginine with fluoride (Yin, 2013a; Yin, 2013b).*



missing, and filled surfaces (DMFS). However, after 2 years of product use, subjects in the 2 test groups using the dentifrices containing arginine had a statistically significant reduction in DMFT increments of 20.5% and in DMFS increments of 19.6% when compared with subjects in the group using the positive control dentifrice with fluoride (Figure 4). Importantly, there were no statistically significant differences with respect to DMFT or DMFS between the 2 test groups containing arginine, showing that both formulations were effective.

Figure 4. *Arginine + Fluoride: superior reduction in caries, 24 month caries clinical*



**Arginine + 1450 ppm MFP versus 1450 ppm NaF, p<0.05*

Test cells: Fluoride= 1450 ppm F as MFP and Fluoride + Arginine = 1.5% Arginine + 1450 ppm F as MFP

Microbiome Studies

A growing number of clinical studies have shown that the key to controlling caries may lie in the ability to foster a microbial community in the oral cavity that is resistant to caries. More specifically, microbes with the Arginine Deiminase System (ADS) have been shown to play a role in caries resilience. Arginine found naturally in the saliva is broken down, primarily by the ADS, which is found in arginolytic bacteria within oral biofilms, to produce ammonia. Ammonia production increases the pH, which inhibits tooth demineralization by neutralizing glycolytic acids and which stabilizes the biofilm community, enabling the survival of the good bacteria or commensals and suppressing the emergence of a cariogenic microflora while also helping to maintain supersaturation conditions for enamel, favoring remineralization. Notably, the ADS has been found to be active in some commensal *Streptococcus* species such as *S. sanguinis* and *S. gordonii*.[18]

It has been known for some time that caries-free individuals have higher pH levels and elevated ammonia levels compared with people with caries—both at rest and after a carbohydrate challenge.[19-22] Arginine serves as a prebiotic, providing nutrition for the beneficial bacteria and weakening the effect of caries-causing bacteria, helping to restore the dysbiotic microflora to a healthy microflora, protecting the caries sufferers. Clinical work by Nascimento explored the impact of adding exogenous arginine on ADS activity, which was measured by the quantification of ammonia produced within oral samples at baseline, after a washout period, after 4 weeks of treatment, and 2 weeks after treatment.[23] In this study, the treatment was a 1.5% arginine-only dentifrice, which was compared to an 1100 ppm fluoride toothpaste in 19 caries-free (DMFT = 0) and 19 caries-active (DMFT > 2) individuals. These studies demonstrated that Arginine increased the ADS activity levels and shifted the flora of caries-active individuals toward a flora more typical of caries-free individuals.[23] More recently, Zheng showed that treatment with a dentifrice containing 8% arginine plus 1450 ppm fluoride helped to normalize the oral microbiome in caries-active individuals.[24] It was found that after treatment, the microbiome of the caries-active individuals became more similar to the caries-free individuals, the enzymatic activity associated with acid production was reduced, and the alkali-generating enzymes and corresponding transcripts were enhanced. Another notable finding from this study was that *S. sanguinis*, which is an ADS-containing bacteria, was enriched, whereas the acidogenic/aciduric *S. mutans* was suppressed. With this important shift in bacteria, the demineralizing capability of the biofilm was substantially reduced.

Caries: Arginine Only Studies

As previously mentioned, there are subsets of the population across the world that would benefit from alternatives to fluoride dentifrices. In certain regions where there is a high level of naturally occurring fluoride, endemic fluorosis remains a problem, while in other parts of the globe, there is a strong consumer preference for anticaries solutions that do not include fluoride. For both these populations there exists a strong unmet need for an effective anticaries dentifrice that is formulated without fluoride. While the body of clinical knowledge on arginine without fluoride is more limited, it is growing. Evidence shows that arginine when formulated without fluoride still exhibits an anticaries effect clinically. With multicenter clinical studies underway in both the United States and China, this body of evidence is certain to expand over time.

The Impact of an Arginine Dentifrice on the Development of Dental Caries in Venezuelan School Children

A double-blind proof of concept study was conducted over a 2-year period to assess the effect of an arginine-containing dentifrice on caries development in 11- to 12-year-old Venezuelan children.[25] A total of 726 children were enrolled in the study, with 601 (304 test subjects and 297 control subjects) completing the study. The test group received a dentifrice containing 1.5% arginine, while the control group used a commercially available sodium fluoride 0.24% (1100 ppm fluoride) dentifrice. All subjects were instructed to brush 3 times a day for 1 minute followed by swishing for 30 seconds with their randomly assigned dentifrice.

Examination of caries prevalence was conducted using the DMFS caries index as the scoring method and the criteria reported by Radike, with the avoidance of any forceful probing of suspected noncavitated pits and fissures for caries lesions.[26] After 6 months, the mean DMFS scores increased only slightly from baseline in both groups: 6.93 ± 0.22 (mean ± SEM) in the control group and 6.59 ± 0.22 in the test subjects. After 1 year, the mean DMFS score in the control group rose to 8.00 ± 0.24, and at 2 years, it leveled off at 7.92 ± 0.30. In contrast, the mean DMFS score in the test group using the arginine dentifrice decreased to 5.50 ± 0.24 after 1 year before rising to 6.99 ± 0.28 at the 2-year time point. Using the method of repeated measures analysis of variance adjusting for baseline values, the DMFS difference between the 2 groups was statistically significant at both the 1-year ($P < 0.001$) and 2-year ($P < 0.024$) time points when looking at premolars and second molars. This study showed that, after 2 years of use, a 1.5% arginine toothpaste was at least as effective as a 0.24% sodium fluoride (1100 ppm fluoride) toothpaste in reducing the formation of caries.

Enhancing ADS Activity through Exogenous Arginine

Beyond toothpastes containing arginine, the effect of introducing exogenous arginine through mint confections on dental caries has also been examined.[27] This second proof of concept study was conducted to determine if a sugarless mint containing an arginine bicarbonate calcium carbonate complex is capable of preventing the development of dental caries in the primary molars and first permanent molars of 10.5- to 11-year-old Venezuelan children. The population enrolled had at least some caries in their primary or permanent teeth as evidence of caries activity. They were all instructed to continue their

daily hygiene regimens with fluoride-containing toothpastes at 1450 ppm fluoride, and they were also exposed to fluoride through the National Salt Fluoridation Program. A total of 200 children were enrolled in this 1-year study, with 100 in the test group administered 2 mints with the arginine bicarbonate calcium carbonate complex twice daily and 100 in the placebo control group administered the sugar-free mints minus the active. The results supported previous findings, showing 75.6% fewer caries in the arginine test group than in the placebo control group of children after 6 months and 50.7% fewer caries after 12 months in the first permanent molars and some early erupting premolars and second molars.[27] (Acevedo, 2008).

These 2 proof of concept studies along with the additive and synergistic benefits demonstrated in the arginine plus fluoride studies were the basis for the large multicenter studies ongoing in the United States and China to determine the efficacy of the active arginine delivered in toothpastes on caries prevention.

Looking Forward/Concluding Thoughts

Caries remains the most common chronic condition faced by humankind worldwide. Fluoride over the past 65 years has been effective at keeping caries rates at lower levels than we might see otherwise, but the caries burden worldwide remains high despite current preventive strategies. Fluoride is delivered in various ways: toothpastes, mouthrinses, varnishes, gels, tablets, and in many water supplies as a public health effort to prevent caries. But it clearly has not resolved the issue, as diet and nutrition are also important in any risk-reduction strategy. The development of additional new actives can also help to alleviate this global oral health issue. Arginine is a new active to use in the battle against caries, with a growing body of evidence both preclinically and clinically for the use of this natural prebiotic to aid in caries prevention strategies.

In addition, addressing the microbiome is an essential element to consider in prevention strategies and when treating caries. Our oral microbiome is acquired at infancy. We know that children with poor oral health and dietary habits are more likely to develop cavities. The development of caries in the deciduous or primary teeth sets the course for the continued development of caries in permanent teeth. A child with an unhealthy microbiome is at risk for oral health problems as an adult. Arginine found within saliva, breast milk, baby formula, and healthy baby foods serves as a natural prebiotic to help maintain a healthy microbiome.

As consumer sentiments change, we must have all the tools available to us to combat caries. As the use of nonfluoride toothpastes, with no known actives to address caries, continues to grow, arginine may be a choice that is equally as effective at preventing caries, albeit by a different mechanism than fluoride. The complementary mechanisms of action of fluoride to strengthen the enamel and arginine to restore the microflora and oral environment to a healthier state have been shown to be a winning combination, demonstrating superior efficacy and the best caries prevention results reported in the literature thus far. Fluoride and a new active arginine offer the practitioner and consumers multiple options, including fluoride alone, arginine alone, or the combination of the 2, based on choice and caries risk. Beyond the exploration of additional new actives to address caries, it is also clear that sugar consumption, poor oral hygiene, and access issues need to be addressed as well in our fight against caries.

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