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The efficacy of combined arginine and probiotics as an add-on to 1450 ppm fluoride toothpaste to prevent and control dental caries in children – A randomized controlled trial

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A R T I C L E   I N F O

Keywords:
Randomized controlled trial
ICDAS
Probiotics
Arginine
Symbiotic
Fluoride toothpaste

A B S T R A C T

Objectives: To determine how daily consumption of a lozenge combining arginine and two probiotic strains affects the Relative Risk Reduction (RRR) in children regarding dental caries transitions and lesion activity at tooth surface level during 10–12 months.

Methods: A total of 21,888 tooth surfaces in 288 children were examined. The intervention group (n = 141) received a lozenge containing 2% arginine, Lacticaseibacillus rhamnosus, LGG® (DSM33156) and Lactobacillus paracasei subsp. paracasei, L. CASEI 431® (DSM33451). The placebo group (n = 147) received a placebo lozenge. Both groups received 1,450 ppm F toothpaste. Primary canines, molars, and first permanent molars were examined clinically (ICDAS0,4) and radiographically (R0,6) at baseline and follow-up. Sealed, filled, and missing surfaces were also included. Caries activity was computed as a sum of each caries lesion’s location, color, texture, cavitation, and gingival bleeding. RRRs were computed with cluster effect on surface level. ICH-GCP was followed, including external monitoring.

Results: A total of 19,950 surfaces were analyzed after excluding 1,938 tooth surfaces. No statistically significant differences were found between the groups. The RRRs showed less caries progression (13.6%, p = 0.20), more regression (0.3%, p = 0.44), and fewer active caries lesions (15.3%, p = 0.15) in the intervention group.

Conclusion: Daily consumption of a lozenge combining arginine and probiotics for 10–12 months given to 5–9-years-old children characterized being with low caries risk demonstrated a marked, though not statistically significant RRR for caries progression, regression, and number of active lesions in the intervention group compared to the placebo-group. ClinicalTrials.gov (NCT03928587).

Clinical significance: Since all the RRRs were in favor of the intervention group and the PF of combined arginine and probiotics is high (81.6%) compared to fluoride toothpaste (24.9%) and arginine-fluoride toothpaste alone (19.6%) the combined pre-and probiotics approach may be a future additional tool regarding caries prevention and control.

1. Introduction

Despite the noticeable decline in caries prevalence since the introduction of fluoridated toothpaste in the 1960s, dental caries remains a public health burden affecting billions of people worldwide [1,2]. Dental caries is the most common disease in the oral cavity; and rank among the top three conditions globally in terms of the highest direct and indirect costs associated with prevention and treatment [3,4].

Dental caries is a preventable imbalance-driven oral disease in the hard tissue of the teeth, occurring when the oral microbiota shifts from a symbiotic balance to dysbiosis [5]. The outcome is determined by the dynamic equilibrium between periods of demineralization and remineralization.
remineralization [6]. Thus, by biofilm control-, limiting sugar intake-, and using fluoridated products such as fluoride containing toothpaste, the demineralization can be prevented (primary prevention) and already active lesions can change to inactive (secondary prevention). [7,8]

In recent years, using pre- and probiotics to modulate the oral ecosystem in order to either preserve (primary prevention) or reestablish (secondary prevention) an oral equilibrium has gained interest [5]. Prebiotics are defined as a "substrate that is selectively utilized by host microorganisms conferring a health benefit" [9]. Arginine is thus classified as a prebiotic, as arginine is metabolized by oral bacteria expressing the Arginine Deaminase System (ADS), resulting in ammonia production, increasing the pH, and thereby reducing tooth demineralization [10]. Probiotics are, conversely, defined as "live microorganisms which, when administrated in adequate amounts, confer a health benefit on the host" [11]. Although the precise mechanisms are not yet fully understood and the effects is strain-specific, adhesion, co-aggregation, growth inhibition, and bacteriocin production are thought to be responsible for the health benefits of probiotics concerning dental caries [12]. There is evidence that arginine and probiotics individually can prevent dental caries [10].

An in vitro study has demonstrated a synergistic effect when arginine and probiotics are combined [13]. Thus, arginine and probiotics may collaborate as a dynamic intervention to restore and thereafter sustain a symbiotic condition if consumed frequently. Arginine and probiotics combined have been proposed as a promising synbiotic, that together with fluoride toothpaste could curtail the dental caries disease [14]. Further, the authors have just published a randomized controlled trial (RCT) supporting a statistically significant effect on caries increment in children aged 5–9 years within an intervention period of 10–12 months. Thus, the null hypothesis stated, there was no significant difference between the intervention and the placebo group concerning progression, regression, and lesion activity on tooth surface level in the mixed dentition of children aged 5–9 years within an intervention period of 10–12 months.

2. Materials and methods

The study was approved by Region Committees on Health Research Ethics, The Capital, Denmark (H-19,002,678), and registered in www.ClinicalTrials.gov (NCT03928587). The comprehensive coverage of the study design, sample size, recruitment, inclusion criteria, randomization, blinding, calibration, clinical examination, and compliance has been provided in Parksen et al., 2023 [15]. Therefore, the materials and methods section will be brief in this paper.

Fig. 1 illustrates the flowchart of the study in terms of enrollment, the randomization and allocation of the 343 children into the intervention (n = 172), and the placebo group (n = 171) (1:1 ratio), dropouts, exclusion and completion of children, and tooth surfaces. Eventually, 141 and 147 children in the intervention and the placebo groups, respectively had completed the double-blinded, parallel-group, placebo-controlled RCT study with an intervention period of 10–12 months. Lozenges with 2 billion CFU in total of the probiotics Lacticaseibacillus rhamnosus, LGG® (DSM33156), Lactobacillus paracasei subsp. paracasei, L. CASEI 431® (DSM33451) and the prebiotic arginine 2% were provided to the intervention group (n = 141) for daily consumption. An

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**Fig. 1.** Flowchart of the randomization, dropout, exclusion, and completion of the children and surfaces in the study.
identical lozenge without arginine or probiotics was provided to the placebo group (n = 147). Parents were advised to brush their children’s teeth twice daily with a provided 1450 ppm fluoride toothpaste.

At baseline and follow-up, a clinical examination (ICDAS, slightly modified) [18] was conducted on all tooth surfaces of primary canines and molars as well as fully, or partially erupted first permanent molar teeth. Additionally, radiographic examination was performed on surfaces that were not sealed, filled, or missing along with the combined clinical and radiographical scores on primary canines and molars, and first permanent molars at baseline. P value calculated with Possion regression (with cluster effect): \( n = 147 \). Parents were advised to brush their children’s teeth twice daily with a provided 1450 ppm fluoride toothpaste.

Overview of clinical registrations on all tooth surfaces (n = 21,888 surfaces in 288 children) and radiographical registrations on occlusal, mesial, and distal surfaces that were not sealed, filled, or missing along with the combined clinical and radiographical scores on primary canines and molars, and first permanent molars at baseline. P value calculated with Possion regression (with cluster effect): \( n = 147 \). Parents were advised to brush their children’s teeth twice daily with a provided 1450 ppm fluoride toothpaste.

**Table 1**

Overview of clinical registrations on all tooth surfaces (n = 21,888 surfaces in 288 children) and radiographical registrations on occlusal, mesial, and distal surfaces that were not sealed, filled, or missing along with the combined clinical and radiographical scores on primary canines and molars, and first permanent molars at baseline. P value calculated with Possion regression (with cluster effect): \( n = 147 \). Parents were advised to brush their children’s teeth twice daily with a provided 1450 ppm fluoride toothpaste.

<table>
<thead>
<tr>
<th>Score</th>
<th>Definition</th>
<th>Intervention (n = 10,716)</th>
<th>Placebo (n = 11,172)</th>
<th>P-value</th>
</tr>
</thead>
</table>
| Caries related | ICDAS_S | Sound | 9748 | 10,056 | 0.30
| | ICDAS_B | Brownish, not wider than 1 mm | 83 | 92 |
| | ICDAS_B1 | Brownish and >1 mm | 24 | 37 |
| | ICDAS_W | Whitis, requiring air drying for 5 s. to be identified | 232 | 223 |
| | ICDAS_Wd | Whitis, seen on a wet surface | 144 | 184 |
| | ICDAS_S2 | Localized enamel breakdown due to caries with no visible dentin | 11 | 18 |
| | ICDAS_S3 | Underlying dark shadow from dentin ± enamel cavitation | 14 | 17 |
| | ICDAS_S4 | Distinct cavity into the dentin extending ≤ 5% of the surface | 17 | 12 |
| | ICDAS_S5 | Extensive distinct cavity into the dentin extending > ½ of the surface | 3 | 2 |
| S / F | | Sealed surface | 41 | 47 |
| m / M | | Filled surface due to caries on primary or permanent tooth | 47 | 75 |
| | | Missing tooth due to caries on primary or permanent tooth | 10 | 15 |
| | | Filled surface due to other reason than caries on primary or permanent tooth | 8 | 14 |
| | | Molar Incisor Hypomineralisation | 156 | 175 |
| | | Missing tooth due to other reason than caries (exfoliation or not erupted) on primary or permanent tooth | 171 | 197 |
| Data | | Data not entered in the eCRF | 0 | 1 |
| Illogical | | Data classified as illogical | 7 | 7 |

**Table 2**

Activity assessment criteria.

<table>
<thead>
<tr>
<th>Activity sum</th>
<th>Definition (score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque stagnation area</td>
<td>Yes = active (1), No = inactive (0)</td>
</tr>
<tr>
<td>Color</td>
<td>White = active (1), Brown = inactive (0)</td>
</tr>
<tr>
<td>Texture</td>
<td>Rough = active (1), Smooth = inactive (0)</td>
</tr>
<tr>
<td>Cavitation</td>
<td>Yes = active (1), No = inactive (0)</td>
</tr>
<tr>
<td>Gingival bleeding</td>
<td>Yes = active (1), No = inactive (0)</td>
</tr>
<tr>
<td>Sealed/Filled/Missing at follow-up</td>
<td>Yes = active (1), No = inactive (0)</td>
</tr>
</tbody>
</table>

*Gingival bleeding was not used for occlusal lesions. If a lesion was recorded individually on the x-ray only gingival bleeding (yes/no) was registered. A lesion was considered active if the activity sum was ≥ 3, except for lesions on occlusal surfaces, for which the threshold was 2 because bleeding is not possible. Radiographical lesions were considered active in case of gingival bleeding. Sealed/Filled/Missing surfaces at follow-up were classified as active since they were most likely active at baseline.

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assessments and the points (0 if no and 1 if yes) allocated to each assessed parameter.

Regardless of participation in the study, the local Public Dental Health Services (PDHS) provided regular oral health care and the staff received written information of the clinical and radiographical findings. Compliance was tracked via weekly electronic Patient Reported Outcomes (ePRO) and phone calls were used to encourage compliance. Unused lozenges were also returned to the sponsor for counting. For adverse events and medication used concomitantly a monthly ePRO was used. The study followed the guidelines of good clinical practice (ICH-GCP) and was externally monitored.

2.1. Data management

Any discrepancies in surface data due to illogical data or input errors were identified and a handling strategy (action) for each incident was carried out as follows:

- Filled surface at baseline but sound or sealed at follow-up. Action: X-rays were used to determine the correct scores (n = 7).
- ICDAS$_5$ at baseline but filled due to other reason than dental caries (fo/FO) at follow-up. Action: The follow-up registration was changed to filled due to dental caries (f/F), since the baseline registration revealed a caries lesion (n = 6).
- ICDAS$_2$ at baseline but Molar Incisor Hypomineralisation (MIH) at follow-up. Action: Both scores were classified as illogical data (n = 1).
- ICDAS$_{4-6}$ or R$_{4-6}$ at baseline but sound, ICDAS$_{1w/b}$, or ICDAS$_{2w/b}$ at follow-up. Action: The scores were classified as illogical for both baseline and follow-up (n = 12).
- MIH at baseline but new caries (NC) at follow-up. Action: The scores were classified as illogical data for both baseline and follow-up (n = 1).
- Multiple missing teeth due to dental caries (m) in the same subject at baseline. Action: If the X-rays revealed naturally exfoliation of the primary teeth, the score m was changed to missing due to other reason (mo/MO), (n = 20).

Table 3
Transition of caries from baseline to follow-up using the highest score from clinical and radiographical registrations presented for Intervention (children = 141) (A) and Placebo groups (children = 147) (B). IC = ICDAS, EBS = Examiner Bias Surfaces, and NTS = Non-Transitional Surfaces.
An activity sum was registered for a surface but without any clinical or radiographical scores. Action: The data was classified as missing (n = 1).

For each surface, both clinical and radiographical registrations were used to determine the highest score at baseline and follow-up. The following rules were enrolled: If a caries lesion was registered with the highest score clinically the ICDAS score was used. If the highest score was detected radiographically, it overruled the clinical registrations. If a caries lesion was registered with the same severity both clinically and radiographically, for example ICDAS 2 and R0, the clinical registration (ICDAS 2) was used. The combined clinical/radiographical score was further merged into the following four categories for simplification and to relate to the routine protocol, commonly used in dental clinics (Table 1, 3): Sound, Brown (ICDAS 1b-2b), Initial (ICDAS 1-2/R1-2), Moderate (ICDAS 3-4/R3-4), and Extensive (ICDAS 5/R5-6). In Table 3, each surface was classified as progressed (Pink area), regressed (Green area), or stable (Blue area), by comparing the baseline and follow-up registrations during the intervention. The term Non-Transitional-Surfaces (NTS) was introduced (Grey area) and used for tooth surfaces that were 1) scored as missing due to dental caries, 2) missing due to exfoliation during the intervention, 3) determined as illogical, or 4) missing data. The NTS were excluded from the data analyzes because they could not be classified as progressed, regressed, or stable. Tooth surfaces scored with MIH or fo/FO were referred to as Examiner-Bias-Surfaces (EBS), (Yellow area).

As seen in Table 2, a lesion was considered active if the activity sum was ≥ 3, except for thresholds on occlusal surfaces for which the threshold was 2, because registration of gingival bleeding was not possible. If an approximal surface was registered as sound clinically but had R1-6 on the radiograph, the lesion was only considered as active in case of gingival bleeding [20]. If a lesion was scored as sealed/filled/missing at the follow-up the surface was classified as active due to the progression (Table 2, 4).

### 2.2. Outcomes using transitions

The outcomes were to define the relative risk reductions (RRR) and the related 95% CI for caries progression, and regression, as well as activity of the caries lesions. Therefore, the following were calculated for the intervention and placebo group: the total number of surfaces which 1) progressed versus the number of surfaces which were stable/progressed, 2) regressed versus progressed/stable surfaces, and 3) active versus inactive caries lesions at the follow-up.

### 2.3. Statistical analyses

Modified Poisson regression with robust variance estimation was used to investigate if baseline and follow-up data at surface level differed between the intervention and placebo group [21]. Generalized estimating equations were used to account for clustering (surfaces within individuals). For these analyzes, the data were grouped into two categories: sound surfaces (ICDAS0) or surfaces with ICDASlow-Ext/sealed/filled/missing. Similar analyzes were done for the radiographical data where the data were grouped in surfaces with no radiolucency (R0), or with radiolucency R1-6. Also, the combined clinical/radiographical score was analyzed for any difference regarding sound (ICDAS0 + R0) or surfaces with Brown, Initial, Moderate or Extensive caries lesions.

The regression coefficients associated to group membership (intervention vs. placebo) were exponentiated in order to obtain the relative risk (RR) values of caries progression, regression, and activity assessment - the latter to reveal if there were any differences in number of active or arrested lesions at the end of the study between the intervention group and the placebo group [16]. The 95% confidence intervals for the RR estimates were computed assuming a normal distribution for the regression coefficients. The relative risk reduction (RPR) was then calculated as 1 minus the RR. Finally, descriptive data about the stable surfaces, and the use of fluoridated tooth paste 1000 ppm F versus 1450 ppm F toothpaste were presented. A p value < 0.05 was considered statistically significant. The IBM SPSS Statistics 28 software was used for all analyzes.

### 3. Results

#### 3.1. Baseline data

A total of 21,888 tooth surfaces in 288 children were examined at baseline and follow-up (Fig. 1). Table 1 illustrates the detailed clinical and radiographical registrations separately and combined for the intervention and placebo group at baseline. There were no statistically significant differences between the groups regarding the clinical categories (Sound versus ICDASlow-Ext/sealed/filled/missing), (p = 0.30), the radiographical categories (R0 versus R1-6), (p = 0.73), or the combined clinical and radiographical categories (Sound/Brown versus Initial/Moderate/Extensive), (p = 0.36).

#### 3.2. Surface transitions

A total of 1,938 tooth surfaces were classified as NTS or EBS (Fig. 1 and Table 3) and were withdrawn from further analyzes; thus, the study material comprised 19,950 tooth surfaces (9,781 in the intervention and 10,169 in the placebo group). Table 3A (intervention) and 3B (placebo) illustrates detailed surface transitions from baseline to follow-up. The surfaces were classified as either progressed (Pink color), regressed (Green color), or stable (Blue color).

The number of stable surfaces was almost equally distributed with 8,537 (87.3%) surfaces in the intervention group and 8,761 (86.2%) surfaces in the placebo group. Most stable surfaces were clinically and radiographically sound/brown (intervention: n = 8,715 (97.8%), and placebo: n = 8,943 (97.2%)).

#### 3.3. Relative risk reduction (RRR)

Caries progression was observed on 535 (5.5%) surfaces in the intervention group, whereas it was 645 (6.3%) surfaces in the placebo group (Table 3 Pink color). The sound surfaces had most frequently progressed to initial (ICDAS1-2/R1-2) caries lesions (intervention: n =...
306 (57.1%), and placebo: n = 351 (54.4%). RRR for progression was determined to be 13.6% (CI: −8.0%–30.9%, p = 0.20) in the intervention group compared to the placebo group.

A total of 339 (3.5%) surfaces in the intervention group and 324 (3.2%) surfaces in the placebo group showed regression (Table 3 Green color). Surfaces with initial lesions (ICDAS\(_{1b/2b}\)) at baseline were most frequently regressed to sound/brown surfaces at the follow-up (intervention: n = 279 (82.3%), and placebo: n = 264 (81.5%)). The RRR for regression was 0.3% (CI: −0.4%–1%, p = 0.44) in favor of the intervention group.

### 3.4. Activity assessment and transitions

Table 4 illustrates the transitions based on the activity assessment between baseline and follow-up examination. A total of 9,701 (intervention group) and 10,048 (placebo group) surfaces were recorded, both at baseline and at the follow-up examination. Of the total number of surfaces 88.0% (8,537 surfaces) and 87.2% (8,761 surfaces) were scored as sound at both examinations in the intervention group and in the placebo group, respectively. Among the surfaces scored as sound at baseline 3.8% (344 lesions) were recorded as active at the final recording in the intervention group and 4.6% (424 lesions) in the placebo group. Among the surfaces scored as active at baseline 49% (279 surfaces) were scored as sound at the final examination in the intervention group, while this was the case for 43% (256 surfaces) in the placebo group. The number of active surfaces at baseline that transitioned to inactive at follow-up was limited to about 5.5% in both groups. Thus, 45.2% (n = 257) of the surfaces in the intervention group versus 51.8% (n = 308) of surfaces in the placebo group were still recorded as active at the follow-up examination.

After dichotomizing the data (Table 4) for both groups to active versus inactive lesions/sound surfaces no significant differences were found between the groups at baseline (p = 0.66). Finally, using the Poisson regression analysis, the RRR was 15.3% (CI: −6.0%–32.4%, p = 0.15).

At baseline 104 and 125 lesions were classified as brown lesions (ICDAS\(_{1w/2w}\)) in the intervention and placebo group, respectively. Nearly 86% of these lesions were classified as inactive at baseline using the sum of activity assessment (Table 2). At the follow-up, 88% of the brown lesions were still inactive.

### 3.5. Fluoridated toothpaste during the study

Among the 288 children 176 (61.1%) used toothpaste with 1450 ppm F\(_{-}\), 106 (36.8%) used toothpaste with 1000 ppm F\(_{-}\), and 6 (2.1%) either did not use fluoride toothpaste or did not know the fluoride concentration at baseline. At follow-up, 259 (89.9%) used 1450 ppm F\(_{-}\) toothpaste, 6 (2.0%) used 1000 ppm F\(_{-}\), and 23 (8.0%) indicated that they were not aware. There was no difference between the groups regarding the use of toothpaste at either baseline or follow-up (p>0.05).

### 4. Discussion

The present study is an extension of the first RCT (published 2023) that investigated the effect of daily intake of a lozenge combining 2% arginine and two probiotic strains versus a placebo lozenge on dental caries increment in children aged 5–9 years [15]. The present analyzes focused on the detailed transitions in terms of caries progression, regression, and lesion activity assessment of the 21,888 tooth surfaces in 288 children, from baseline to follow-up.

In both the main study [15] and in the present study the clinical assessments of lesion severity was assessed by the highly validated ICDAS system [18]. The radiographical assessment of lesion severity was assessed by the system suggested by Ekstrand et al. [17] but in this case extended to seven scores as it could be compared with the ICDAS seven score system. The present study also focused on lesion activity assessment for both baseline and follow-up and for those assessments the components of the ICDAS-LAA system was used but differed with the points and the gingival bleeding. The validation of the ICDAS-LAA system in terms of reproducibility and accuracy has been evaluated in two systematical reviews [8,18] and in both cases the ICDAS-LAA system came out as one of the best and judged as moderately reproducible and accurate.

The activity assessment revealed that <20% of all brown lesions (ICDAS\(_{1b/2b}\)) were active at baseline opposite to >90% for the white initial lesions (ICDAS\(_{1w/2w}\)) in both groups. Thus, the sound/brown surfaces were merged in the combined clinical and radiographical analysis at baseline. It should be emphasized that the color of the lesions was one of the criteria for the activity assessment. Future studies utilizing ICDAS may find it interesting to handle brown lesions independently from white initial lesions.

It is well known that arginine increases the pH in biofilms, which reduces tooth demineralization [10]. On the other side, probiotics possess the ability to displace more cariogenic pathogens [10]. Thus, arginine and probiotics may have both a regulating and preventive impact on dental caries control by affecting and maintaining an oral equilibrium. According to meta-analyses, arginine and probiotics are individually capable of affecting caries increment [22,23]. Though not significant our data demonstrated that the intervention group experienced lower rate of progression (RRR: 13.6%), along with fewer active caries lesions (RRR: 15.3%). These findings indicates that a lozenge combining prebiotic arginine and probiotics may affect caries in a positive pattern. It was unexpected that the combination of arginine and probiotics also resulted in a little higher regression rate (RRR: 0.3%). The administration of probiotics in young children with high caries risk has been linked to statistically significant (p = 0.03) higher rate of caries regression [24]. Additionally, a recent in vitro study demonstrated that fluoride (2000 ppm), arginine (2%), and LGG\(^\text{®}\) combined, enhanced enamel remineralization [25]. When the three components were combined, the mineral density determinant (regression) was shown to be significantly higher, even than arginine and fluoride together, indicating the potential for arginine and probiotics as a supplement to fluoride toothpaste [25]. Thus, the findings in the present analyzes of the first RCT study in vivo with combined arginine, probiotics, and fluoride seems to support the findings in vitro.

The individual differences between the groups did not reach statistical significance (p> 0.05); hence, the null hypotheses could not be rejected. However, as all the RRRs did point in the same direction, explicitly in favor of the intervention group, should be seen as a strength of the study and its results regarding the possible benefit from combined arginine and probiotics in caries prevention and control in practice. In contrast to Porsken et al. [15], the data in the current study were handled blinded due to post hoc analyzes. The limitations of the present study were otherwise like those described in the main paper [15]. Most of the caries lesions were found in the initial category at baseline (Table 3). According to Vanders et al. [1], only approximately 1/3 of the initial caries lesions in the primary teeth were expected to progress over 12 months [26]. With an intervention time of 48 months up to 2/3 of the initial lesions in both primary and permanent teeth might have progressed [26]. Thus, the duration of the trial may have been too short to achieve a full effect. Additionally, the progression rate was small due to the low caries risk group in this study which probably is the one of the main reasons for the lack of significance. As in individuals with low to moderate caries risk, it is challenging to demonstrate a greater anti-caries effect [27]. Further, the analyzes in the present study are most likely underpowered as the sample size was estimated for the primary aim (Δ mean\( \Delta \text{ICDAS}_b\)) of the main study [15] and neither progression or regression of dental caries, nor activity of caries lesions. The examiners were theoretically trained, but not clinically calibrated nor trained in the ICDAS-LAA as it was not one of the endpoints in the main paper. As a result, the activity assessment may have been impacted. Even if the results of such activity assessments can be thought...
of as surrogate measures that do not always correlate directly with the clinically outcome, they may provide useful information [8]. Not considering the activity of a caries lesion potentially could lead to unnecessary treatments that may be iatrogenic [8]. By utilizing the parameters outlined in the ICDAS LAA, it becomes possible to determine whether a caries lesion is active or inactive at each dental visit. Thus, it seemed reasonable to also elaborate on the activity assessment as progression only can be evaluated over time. Yet another limitation was that the examiner was blinded to the baseline registrations. If data regarding treatment performed at the PDHS during the study had been obtained it would be beneficial, as it is unknown why a surface was treated in the PDHS. In the present study the sealed surfaces were considered as progressed, but it is likely that it was due to prophylactically caution. The MIH surfaces were excluded from the analyzes due to EBS, which could have been avoided if the two examiners also were calibrated in MIH.

The children received 1450 ppm F toothpaste during the intervention as a part of the study. The parents were instructed to brush their child’s teeth with the toothpaste twice a day and at least 30 min before or after intake of the lozenges, as the activity of the probiotic strains was thought to be inhibited by fluoride. However, an in vitro study has found that NaF does not inhibit LGG® [28]. At baseline about 60% of the children had their teeth brushed with 1450 ppm fluoridated toothpaste while about 90% of the children used 1450 ppm F toothpaste at follow-up. The differences in use of F toothpaste at baseline and the final examination were not significant (p > 0.05). The use of fluoridated toothpaste with different concentrations did not seem to have influence the observed transitions. However, those children who changed from 1000 to 1100 ppm F toothpaste at baseline to 1450 ppm F during the study would have gained a preventive effect as the mean preventive fraction (PF) is 9.7% when comparing daily use of 1000–1100 ppm F with 1500 ppm F toothpaste [1]. The fluoride toothpaste may have contributed to the regression (remineralization) as found in the in vitro study described above, but this direct effect cannot be confirmed in the present study.

No other RCT has yet been conducted with combined arginine and probiotics, thus direct comparisons are difficult. As these analyzes are an extension of the main study, which did reach statistical significance (p = 0.007), data from the main study is used to address the clinical relevance regarding combined arginine and probiotics. As the preventive fraction is calculated by the difference in caries increment between the intervention and placebo group, divided by the mean caries increment in the placebo group, the PF of the lozenge was 81.6% in the primary dentition [15]. The use of fluoridated toothpaste versus not using fluoride toothpaste is found to have an average PF of 24.9% in both dentitions [2]. According to other probiotics studies conducted in children with a statistically significant efficacy on caries increment the PF ranges between 35.4%–81.3% with a duration time between 3 and 21 months [29–33]. The PF of 81.3% was found in the study with the longest duration time (21 months) and with the addition of 2.5 mg fluoride/L to the intervention group [33]. For 1.5% arginine toothpaste the PF on DMF-S after 2 years varies between 16.5–19.6% [34,35]. Based on the PFs mentioned above and the RRRs in the present analyzes it appears that combined arginine and probiotics as an addition to 1450 ppm fluoride toothpaste is more effective as caries prevention and control than probiotics, arginine toothpaste, or fluoride toothpaste alone. However, it should be tested in future studies. Because the arginine in the mouth is at its highest 2 h versus 1.5%) and the data is in different dentitions, it is a mix of two probiotics strains, and the administration form is a lozenge which could be important for the colonization abilities, all of which could explain why it may have shown a greater potential.

With the use of arginine and probiotics as caries management, concern has been raised regarding potential side effects. For arginine the concern is that it could create an alkalization-related shift of the oral biofilm towards dominance of anaerobe oral pathogens such as P. gingivalis favoring the development of periodontal disease [36]. However, the addition of probiotics might counter the concerns for arginine as probiotics are thought to inhibit the growth of oral pathogens [25]. Further studies concerning the mentioned side effect of arginine are needed. But in a preliminary agar test system, conducted by the sponsor, LGG® and L. CASEI 431® were shown to competitively exclude oral pathogens including P. gingivalis and S. mutans (internal data / unpublished 2007). Further, no difference between the groups in mean gingivitis was observed in the main study [15]. From a cariogenic point of view the use of lactobacilli as probiotics seems controversial as they are generally viewed as cariogenic [37]. But studies have evaluated that the acidogenicity in the dental biofilm seems to depend on the strain and type of sugar available [13,14]. Furthermore, arginine has the potential to affect the lactic acid production from the probiotics by increasing the pH value [25]. Lastly, the main study showed that dental caries decreased significantly and did not enhance in the intervention group, being similar to other probiotics studies, why the two strains L. CASEI 431® and LGG® do not appear to be of cariogenic concern [10, 15]. So, in present time there does not seem to be a compelling reason to halt the symbiotic caries prevention and management concept from being further developed. The above concerns along with the PF previously described could actually indicate beneficial value of using the combined concept rather than the separately use of arginine or probiotics in an oral context.

The use of combined arginine and probiotics is still in its infancy, and more studies are required to explore the ideal route of administration, frequency in use, accurate dose, and strains along with the long-term effect or possible side effects before it could be implemented as an evidence-based caries preventive strategy.

5. Conclusion

In conclusion, the additionally analyzes of daily consumption of combined arginine and probiotics among healthy children with low caries risk revealed that over a 10–12-month study period the children in the intervention group did experience lower caries progression rate, a slightly higher caries regression rate and a lower number of active lesions, but none of them reached a significant level. Future RCT studies with longer intervention time and various caries risk groups may provide valuable insights into the long-term efficacy of the present approach, as avenues for more effective strategies for prevention and control of dental caries need to emerge.

CRediT authorship contribution statement

Camilla Juhl Parksen: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Project administration.
Kim Rud Ekstrand: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Project administration, Supervision.
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Tove Larsen: Conceptualization, Writing – review & editing, Supervision.
Luis Eduardo Garrido: Formal analysis, Writing – original draft, Writing – review & editing.
Azam Bakshandeh: Conceptualization, Methodology, Software, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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