

HUMAN RANDOMIZED CONTROLLED TRIAL

# Consumption of *Lactobacillus reuteri*-containing lozenges improves periodontal health in navy sailors at sea: A randomized controlled trial

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**Abstract****Background:** The objective of this trial was to evaluate whether the regular consumption of probiotics may improve the known deterioration of periodontal health in navy sailors during deployments at sea.**Methods:** 72 healthy sailors of a naval ship on a practicing mission at sea were recruited and randomly provided with a blinded supply of lozenges to be consumed twice daily for the following 42 days containing either the probiotic strains *Lactobacillus reuteri* (DSM 17938 and *L. reuteri* (ATTC PTA 5289) (test  $n = 36$ ) or no probiotics (placebo  $n = 36$ ). At baseline, at day 14 and day 42 bleeding on probing (primary outcome), gingival index, plaque control record, probing attachment level, and probing pocket depth were assessed at the Ramfjord teeth.**Results:** At baseline there were no significant differences between the groups. At day 14 and day 42 test group scores of all assessed parameters were significantly improved ( $P < 0.001$ ) compared to baseline and to the placebo group which by contrast showed a significant ( $P < 0.001$ ) deterioration of all parameters at the end of the study.**Conclusions:** The consumption of probiotic *L. reuteri*-lozenges is an efficacious measure to improve and maintain periodontal health in situations with waning efficacy of personal oral hygiene.**KEYWORDS**

gingivitis, oral hygiene, probiotics

## 1 | INTRODUCTION

According to current etiological concepts the dysbiotic overgrowth of virulent inflammophilic pathobionts within the sulcular microbiota is an essential step for the clinical manifestation of gingivitis and periodontitis.<sup>1</sup> Strengthening their microbial competitors by the targeted consumption

of probiotics may therefore have the potential to prevent or alleviate dysbiosis-associated inflammations even in the absence of efficacious mechanical plaque control. According to a definition set by a joint expert group of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations probiotics are “live microorganisms which, when administered in adequate

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amounts, confer a health benefit on the host".<sup>2</sup> They may directly interfere with the adhesion, growth, and metabolism of competing pathogenic bacterial species or may improve the efficacy of the host response by interfering with cellular elements of the mucosal immune system or the function of mucosal epithelial barriers.<sup>3</sup>

Based on data from numerous investigations probiotic strains of *Lactobacillus reuteri* appear to be particularly suited for being used in the evaluation of such an alternative strategy in the control of gingivitis. In *in vitro* experiments they inhibited the growth of a variety of bacterial periodontopathogens and oral *Candida* species by the release of specific bacteriocines, hydrogen peroxide or organic acids.<sup>4,5</sup> Further animal studies identified a host of immunomodulatory interactions between probiotic *L. reuteri* strains and the mucosal immune system, affecting targets as diverse as t-cell maturation,<sup>6</sup> inhibition of proinflammatory cytokine production,<sup>7</sup> and the upregulation of healing-promoting plasma levels of oxytocin.<sup>8</sup>

Randomized controlled trials evaluating the impact of *L. reuteri* consumption on clinical and microbiological parameters of gingival and periodontal health in individuals affected by gingivitis or periodontitis however presented diverging results. Although several of them reported a major *L. reuteri*-associated reduction of gingival and periodontal inflammation<sup>9-11</sup> or a significant enhancement of pocket depth reduction<sup>12</sup> others failed to identify any beneficial effect on clinical and microbiological parameters<sup>13</sup> or detected only a significant impact on the composition of the oral microbiota.<sup>14,15</sup>

In all of the trials lozenges or a chewing gum containing a combination of the two probiotic *L. reuteri* strains ATCC PTA 5289 and DSM 17938/ATCC 55730 were used. ATCC 55730 was replaced in the more recent trials by DSM 17938, which is a daughter strain to ATCC 55730 with identical probiotic properties but without antibiotic resistance gene carrying plasmids.<sup>16</sup> The selection of study participants however was quite diverse, ranging from healthy younger persons<sup>9,13,14</sup> and healthy pregnant women<sup>10</sup> to periodontitis patients,<sup>12</sup> diabetics,<sup>11</sup> and frail elderly.<sup>15</sup> Further the applied study designs were heterogenous. They comprised a cross-over model of experimental gingivitis,<sup>13</sup> as well as the consumption of *L. reuteri* as an adjunct to scaling and root planing,<sup>12</sup> as an adjunct to professional mechanical plaque removal<sup>14</sup> or as a sole intervention without concomitant mechanical plaque control measures or hygiene instructions.<sup>9,10,15</sup>

Next to those obvious differences also insufficiently controlled hidden confounders of gingivitis development like diet<sup>17</sup> or stress-induced cortisol levels<sup>18</sup> may have contributed to the inconclusive evidence derived from the current set of available clinical trials. It was therefore a central intention of this controlled clinical trial to reduce the confounding impact of diverging general health, diet and environmental factors on

the study results by performing it in study participants only with a clinically and serologically verified status of general health and to standardize their living conditions as well as their general diet during the experimental observation period.

Because of the specific conditions of their professional environment navy sailors may meet many of those requirements for an ideal study cohort. Following established standards German navy sailors are obliged to pass standardized medical and dental examinations for the verification and documentation of their dental and general health fitness status before entering a naval ship for a mission at sea.<sup>19</sup> At sea they are forced to live in the confinements of the ship and have to follow a standardized schedule of work duties and leisure-time. In this period also the variety of their daily diet is restricted because of a limited number of main meals offered by the galley.

Further, a systematic survey identified German navy sailors and other soldiers of the German Armed Forces as a susceptible gingivitis and periodontitis risk group.<sup>20</sup> This is reflected by the results of a longitudinal trial assessing the oral health status of 134 German soldiers aged 20 to 54 during a 5-month peace-keeping mission in Bosnia-Herzegovina.<sup>21</sup> They documented a significant deterioration of oral hygiene efficacy accompanied by a significant increase in the prevalence of gingivitis-associated Periodontal Screening and Recording Index (PSR) scores.<sup>22,23</sup>

It was therefore the purpose of this controlled clinical trial to evaluate whether the regular consumption of lozenges containing the probiotic *L. reuteri*-strains DSM 17938 and ATCC PTA 5289 is able to prevent an expected deterioration of periodontal health or even to improve it in medically healthy crew members of a frigate of the German Navy during a military practice mission at sea.

## 2 | MATERIALS AND METHODS

The investigation was designed as a prospective, two arm parallel group double-blind placebo-controlled randomized trial. The study protocol was established in accordance with the declaration of Helsinki and the criteria of good clinical practice. It was approved by the ethics committee of the University of Wuerzburg (file # 81/16-ge) and was registered with ClinicalTrials.org (identifier no: NCT02775019). All participants had signed a written informed consent before their inclusion.

### 2.1 | Study population

The study population was recruited from 204 healthy crew members of the German Navy frigate "Brandenburg" passing the routine North Atlantic Treaty Organization (NATO) standardization agreement (STANAG) 2466 dental fitness examination with a dental fitness class score of 1 (dentally fit) or



2 (condition expected not to cause a problem within the next year)<sup>19</sup> before boarding the ship for a 6-week practicing mission at sea. As all of them were actively involved in a military practicing mission at sea, evaluation time per study participants was restricted and thus recording of the study parameters had to be confined to the Ramfjord teeth. Before screening and recruitment all crew members were comprehensively informed about the aims and involved risks of study participation followed by individual face to face discussions.

Inclusion criteria were age 18 to 65 years, a minimum of 12 remaining teeth, the presence of the Ramfjord teeth (16, 21, 24, 36, 41, 44)<sup>24</sup> or their replacements and at least one Ramfjord tooth showing bleeding on probing (BoP).

Exclusion criteria were the regular intake of antiinflammatory medications possibly interfering with gingival inflammation, the regular use of antimicrobial mouthrinses, gels or comparable medications, the use of antibiotics  $\leq 4$  weeks before study participation, the presence of systemic disease interfering with gingival health (e.g., diabetes), and a history of alcohol or drug abuse.

## 2.2 | Sample size calculation

Sample size calculation was performed for the primary outcome variable (BoP-positive sites). Based on the findings of two preceding surveys evaluating the oral health of German soldiers<sup>20,21</sup> a mean number of  $18 \pm 9$  (SD) BoP-positive sites in the placebo group at the end of the study was assumed. To be able to verify a difference  $\geq 50\%$  in comparison to the test group with a given power of 0.9 and  $P \leq 0.05$  a required minimal sample size of  $2 \times 27$  participants was calculated. Because of expected drop-outs and protocol violations it was decided to recruit a total of  $2 \times 36$  participants for the trial.

## 2.3 | Primary study outcome and null hypothesis

The primary study outcome of this trial was the observed percentage of gingival probing sites at the Ramfjord teeth being positive for BoP. At each Ramfjord tooth six sites were evaluated (mesio-buccal, buccal, disto-buccal, disto-oral, oral, mesio-oral) by inserting a disposable manual periodontal probe (sterile oral examination kit; Kerr Biberach, Germany) to the bottom of the gingival sulcus or periodontal pocket. The application of a probing force of  $\approx 0.2$  N had been calibrated before using a letter scale. Any bleeding spot appearing within 30 seconds after probing was recorded as a BoP-positive site. The null hypothesis of this trial was the absence of a significant difference in the percentage of BoP-positive probing sites between test and placebo group at the end of the 42-day observation period.

## 2.4 | Secondary study outcomes

Secondary study outcomes were the modified gingival index (GI),<sup>25</sup> the plaque control record (PCR),<sup>26</sup> probing pocket depth (PPD), and probing attachment level (PAL). PPD and PAL were recorded to the nearest millimeter concomitantly with BoP at identical sites.

GI scores were recorded at the buccal aspects of teeth 16, 41, 44 and at the oral aspects of teeth 21, 24, 36.

## 2.5 | Sequence of study examinations

At day 0 (baseline) BoP, PPD, PAL, GI, and PCR were recorded in all study participants.

Subsequently a supply of experimental lozenges (test and placebo) sufficient for the next 6 weeks was handed out to them. They were not informed about their dental status and did not receive any oral hygiene instruction or practical training. At day 14 (intermediate reevaluation) and at day 42 (final examination) the recording of BoP, PPD, PAL, GI, and PCR was repeated.

## 2.6 | Experimental lozenges

The experimental lozenges were manufactured by BioGaia AB (Lund, Sweden) and consisted of isomalt (filler), hydrogenated palm oil, peppermint flavor, menthol flavor, peppermint oil, and sucralose. They were identical to a commercially available product\*. Test lozenges contained  $\geq 1 \times 10^8$  colony forming units each of *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 whereas placebo lozenges were void of probiotics.

## 2.7 | Consumption compliance

The study participants were instructed to consume the assigned lozenges twice daily during the 42 day observation period. Consumption compliance was calculated by counting the remaining lozenges in the assigned lozenge-containing bottles brought back to the reevaluation visits.

## 2.8 | Nutritional status

The choice of main meals to be consumed by the study participants during the observation period was limited to a small variety served by the galley of the ship. Individual intake of sweet snacks was documented by a self-reported sugar consumption questionnaire.

## 2.9 | Calibration, randomization, and blinding

All examinations were performed by one dentist (J.R.), who was blinded to the randomization of the study participants.

\* Prodentis lozenges, BioGaia AB, Lund, Sweden.



The recording of the study outcomes was trained and calibrated in practical training sessions and discussions with the coauthors before the onset of the study. Test and placebo lozenges were of identical shape, weight, and flavor and packed in neutral plastic bottles displaying a unique identifier number only. The distribution of the lozenges to the study participants was performed by a study nurse not involved in the clinical assessment of the study participants and followed the sequence of a computer-generated randomization list with a block size of 4, being stratified for smoking.

### 2.10 | Adverse events

The study participants were instructed to report any adverse events (AE) related or unrelated to the consumption of the lozenges immediately and were explicitly interviewed for the occurrence of AE at the reevaluation visits at days 14 and 42.

### 2.11 | Statistical analysis

The modalities of the statistical analysis were fixed in writing (see appendix) before unblinding the trial data. It was consented to perform an intent to treat (ITT) analysis following the principles of “last observation carried forward” (LOCF), which included not only participants with complete data sets but also those who did not finish the study but who at least were reevaluated once at day 14. To verify the validity of the results based on the ITT LOCF-analysis a further ITT analysis was performed evaluating only study participants with complete data sets. Wilcoxon signed-rank test was used to evaluate differences within the groups, analysis of covariance (ANCOVA) or Mann-Whitney *U* test was used to assess differences between the groups.

Statistical analysis was not adjusted for potential confounders as age, BMI, smoking habits, education level, and military rank were evenly distributed across both experimental groups. Further, the mandatory medical examination for the verification of mission fitness before boarding the ship ruled out the presence of serious systemic disease like diabetes, etc.

The level of significance was set to  $P \leq 0.05$ . The software used for the statistical analysis was: SPSS version 23 (IBM Corp., Armonk, NY, USA.)

## 3 | RESULTS

### 3.1 | Recruitment, drop-outs, protocol violations

Recruitment and drop-outs are depicted as a CONSORT<sup>27</sup> flow diagram in Figure 1. One hundred eighty one out of a total of 204 crew members with a verified STANAG 2466 dental fitness score  $\leq$  class 2 met the inclusion criteria of this

trial. One hundred twenty four of them also volunteered for study participation. Finally 72 of those eligible volunteers displaying the highest prevalence of gingival inflammation were included in the trial. Thirty-six individuals were randomly assigned to the test, another 36 to the placebo group. Between baseline and final examination at day 42 a total of six participants of the test group and four of the placebo group had left the study prematurely because of withdrawal of consent for study participation (4), suffering diseases or trauma injuries unrelated to the consumption of the experimental lozenges (5) or for personal reasons (1). Finally a total of 62 individuals (30 test group, 32 placebo group) was evaluated in the final examination at day 42. Five of those finished the trial with protocol violations. Three of them (1 placebo/2 test) reported, that they had stopped consuming the lozenges during the final 3 weeks of the trial. Further, two placebo group members had received systemic antibiotics between day 14 and the final examination at day 42 because of emerging health problems unrelated to the consumption of the lozenges.

### 3.2 | Reporting of adverse events

During the course of the trial no AE were reported by the study participants.

### 3.3 | Consumption compliance

At the first reevaluation at day 14 the calculated mean numbers of consumed lozenges were  $23.6 \pm 4.4$  (SD) (test group) versus  $23.4 \pm 4.0$  (SD) (placebo group). At the final reevaluation at day 42 these figures increased to  $70.0 \pm 13.3$  (SD) consumed lozenges in the test group versus  $67.1 \pm 15.1$  (SD) consumed lozenges in the placebo group, indicating overall a good compliance with the study protocol.

### 3.4 | Health and social profile of the study participants

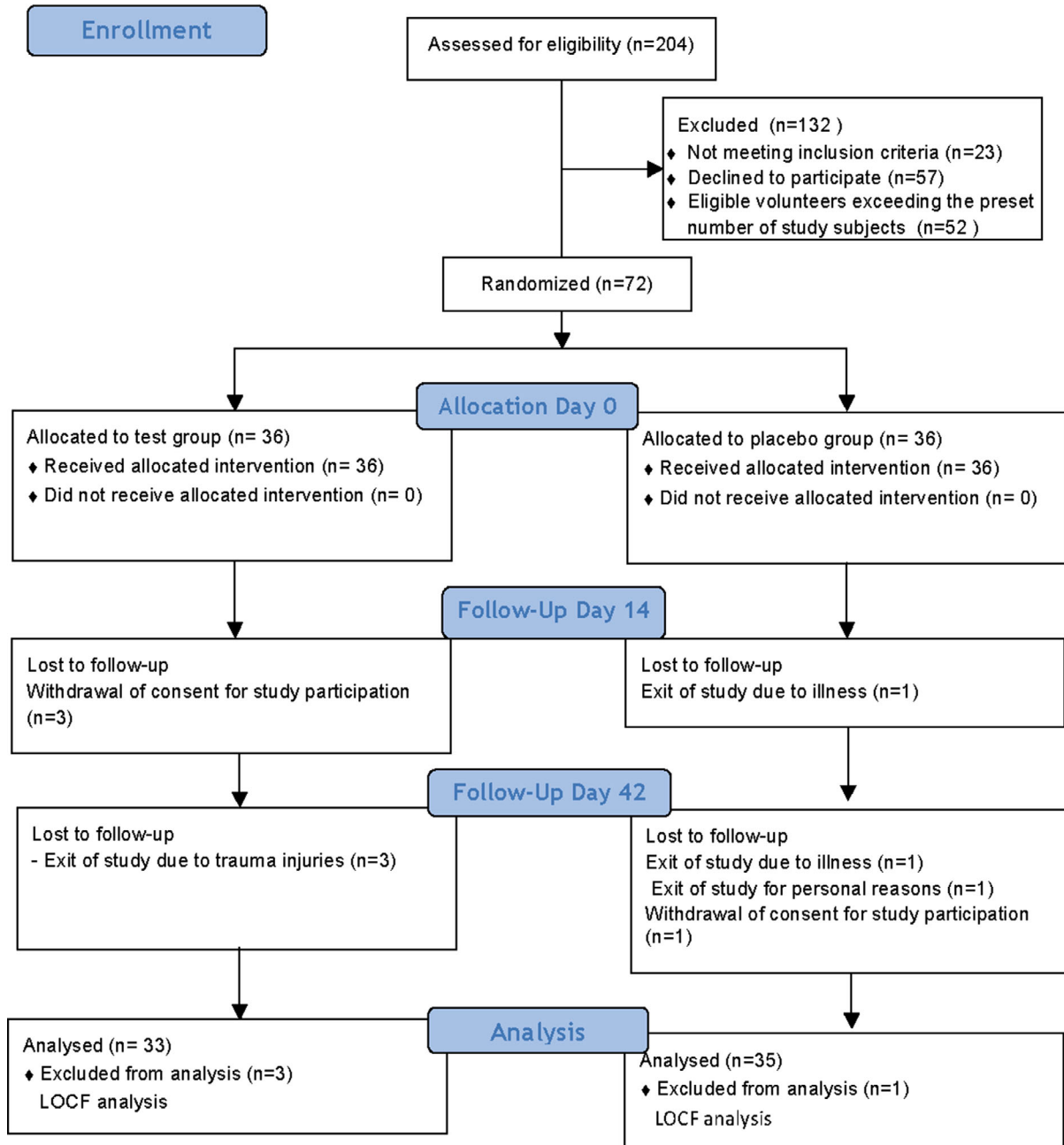
The health and social profile of the study participants is shown in Table 1. The majority of them (94%) were male, the mean age was  $27.0 \pm 5.0$  years. All of them had a health status meeting military standards for active crew members of naval ships as verified by a mandatory medical examination performed before boarding the ship. It is noteworthy however that the observed mean BMI score in both groups was at the upper limit of the normal range and that the majority (74%) of the study participants were regular smokers. Sixty percent of the study participants had the rank of a petty officer, reflected by the higher track secondary school education as the most frequently observed education level. All observed differences between both groups regarding health and social profile were small and statistically not significant.



# CONSORT

TRANSPARENT REPORTING of TRIALS

## CONSORT 2010 Flow Diagram



**FIGURE 1** Recruitment, drop-outs, and protocol violations during the study observation period

### 3.5 | Percentage of BoP-positive sites (primary study outcome)

The observed percentage of BoP-positive probing sites in the evaluated Ramfjord teeth are presented in Table 2. In the LOCF analysis the baseline percentage of BoP-positive

sites did not differ significantly between test and placebo group ( $41\% \pm 22\%$  versus  $37\% \pm 20\%$ ). At the subsequent reevaluation visits the percentage of BoP-positive sites in the test group significantly shrunk to  $14\% \pm 13\%$  at day 14 ( $P < 0.001$ ) and to  $10\% \pm 13\%$  at day 42 ( $P < 0.001$ ) when



**TABLE 1** Health and social profile of the study participants included in the LOCF analysis

Variable	All participants <i>n</i> = 68	Placebo group <i>n</i> = 35	Test group <i>n</i> = 33
Age [years]—mean (SD)	27.0 ( $\pm$ 5.0 SD)	26.8 ( $\pm$ 4.4 SD)	27.3 ( $\pm$ 5.6 SD)
Female sex—no. (%)	4 (6%)	2 (6%)	2 (6%)
Body mass index [kg/m <sup>2</sup> ]—mean (SD)	25.6 ( $\pm$ 2.7 SD)	25.8 ( $\pm$ 2.7 SD)	25.4 ( $\pm$ 2.7 SD)
<sup>a</sup> Regular smoker—no. (%)			
No	18 (26%)	9 (26%)	9 (27%)
Yes, $\leq$ 5 pack-years	19 (28%)	9 (26%)	10 (30%)
Yes, >5 pack-years	31 (46%)	17 (49%)	14 (42%)
<sup>b</sup> Education level—no. (%)			
Secondary school, lower track	9 (13%)	5 (15%)	4 (12%)
Secondary school, higher track	44 (66%)	23 (68%)	21 (64%)
University-entrance qualification	14 (21%)	6 (18%)	8 (24%)
Not specified	1 (1%)	1 (3%)	0
Military rank—no. (%)			
Seaman	24 (35%)	12 (34%)	12 (36%)
Petty officer 1st, 2nd or 3rd class	41 (60%)	22 (63%)	19 (57%)
Officer	3 (4%)	1 (3%)	2 (6%)

SD, standard deviation.

Percentages of categories of a variable may not add up to 100% because of rounding.

<sup>a</sup>Smoking: A pack-year of cumulative smoking dose means smoking one pack of cigarettes for 1 year or an equivalent of other tobacco products.

<sup>b</sup>Education: The German education system secondary school has two tracks of the secondary school, "Hauptschule" (lower) and "Realschule" (higher).

**TABLE 2** Percentage of sites positive for bleeding on probing (BoP)

Visit	Placebo group <i>n</i> = 35	Test group <i>n</i> = 33	Difference between groups
<b>Intent to treat LOCF analysis</b>			
Baseline mean $\pm$ SD	37 $\pm$ 20	41 $\pm$ 22	
Day 14 mean $\pm$ SD	34 $\pm$ 17	14 $\pm$ 13	
Difference baseline-day 14 mean (95% CI)	-4 (-7 to +0)	-27 (-33 to -22)	-22 (-27 to -17)
<i>P</i> between groups			<0.001
<i>P</i> within groups	0.08	<0.001	
Day 42 mean $\pm$ SD	42 $\pm$ 17	10 $\pm$ 13	
Difference baseline-day 42 mean (95% CI)	+5 (-0 to +10)	-31 (-38 to -25)	-34 (-40 to -28)
<i>P</i> between groups			<0.001
<i>P</i> within groups	<0.001	<0.001	
<b>Intent to treat analysis</b>			
Baseline mean $\pm$ SD	37 $\pm$ 19	40 $\pm$ 22	
Day 42 mean $\pm$ SD	43 $\pm$ 17	10 $\pm$ 13	
Difference baseline-day 14 mean (95% CI)	+5 (+0 to +11)	-30 (-37 to -24)	-34 (-27 to -17)
<i>P</i> between groups			<0.001
<i>P</i> within groups	0.05	<0.001	

Comparisons between the groups: Analysis of covariance.

Comparisons between the groups: Wilcoxon signed-rank test.

compared to baseline. In the placebo group by contrast the percentage of BoP-positive sites observed at day 14 ( $34\% \pm 17\%$ ) remained almost unchanged and was significantly ( $P < 0.001$ ) increased ( $42\% \pm 17\%$ ) at day 42 when compared to baseline. At day 14 and at day 42 the observed differences between both

experimental groups proved to be significant ( $P < 0.001$ ). Reanalyzing the primary study outcome by an additional ITT analysis limited to study participants finishing the study with complete data sets replicated and confirmed the results of the ITT LOCF-analysis. The null hypothesis of no



differences between both experimental groups at day 42 was rejected.

### 3.6 | Secondary study outcomes

#### 3.6.1 | Gingival index, plaque control record

The analysis of recorded GI and PCR scores are shown in Table 3. At baseline mean GI scores did not differ significantly between the groups (GI placebo group  $1.3 \pm 0.4$  versus GI test group  $1.3 \pm 0.5$ ). At day 42 the observed mean GI score of the placebo group had significantly ( $P < 0.001$ ) increased to GI  $1.5 \pm 0.4$  whereas in the test group it significantly ( $P < 0.001$ ) decreased to GI  $0.5 \pm 0.4$  when compared to baseline values. Differences between the groups at day 14 and at day 42 were significant ( $P < 0.001$ ).

Baseline mean PCR scores did also not differ significantly between the groups ( $88\% \pm 11\%$  placebo group versus  $83\% \pm 11\%$  test group). At day 42 the recorded mean PCR score of the placebo group had significantly ( $P = 0.003$ ) increased to  $94\% \pm 10\%$  whereas in the test group it significantly ( $P = 0.006$ ) decreased to  $75\% \pm 15\%$  when compared to baseline values. Differences between the groups at day 14 and day 42 were significant ( $P < 0.001$ ).

#### 3.6.2 | Probing pocket depth, probing attachment level

The results of the PPD and PAL assessments are displayed in Table 4. At baseline mean PDD as well as mean PAL values did not differ significantly between the groups ( $1.7 \pm 0.4$  mm PPD placebo group versus  $1.7 \pm 0.6$  mm PPD test group;  $1.8 \pm 0.5$  PAL placebo group versus  $1.8 \pm 0.6$  PAL test group). At day 42 the observed mean PPD of the placebo group had increased significantly ( $P < 0.001$ ) to  $2.0 \pm 0.5$  mm whereas in the test group it had decreased significantly to  $1.5 \pm 0.4$  mm ( $P < 0.001$ ) when compared to baseline. At day 42 also the observed mean PAL of the placebo group had increased significantly ( $P < 0.001$ ) to  $2.1 \pm 0.5$  mm whereas in the test group it had decreased significantly ( $P < 0.001$ ) to  $1.6 \pm 0.4$  mm when compared to baseline. At day 14 and at day 42 all observed differences between the groups regarding PPD and PAL were significant ( $P < 0.001$ ).

A subanalysis of the PPD and PAL data comparing study participants with gingivitis (PSR score = 2) to those suffering from periodontitis (PSR score  $\geq 3$ ) as verified by the STANAG 2466 dental fitness examination is shown in Table 5. It confirms the observations made for the total study cohort and shows a significant reduction of PPD and PAL in the test group over the 42 day study period for study participants with chronic gingivitis and those with periodontitis alike. In the placebo group again a significant deterioration of recorded PPD and PAL scores is evident in both subgroups over the course of the study.

## 4 | DISCUSSION

The significant increase of observed BoP, GI, PCR, PPD and PAL scores in participants of the placebo group at the end of the 42 day observation period of this trial confirms the findings of other studies reporting a significant deterioration of periodontal health in active duty personnel during military missions.<sup>19,21,28</sup> Members of the test group by contrast, regularly consuming the probiotic *L. reuteri*-containing lozenges, were not affected by any deterioration of the evaluated primary and secondary study outcomes. At day 42 all assessed parameters were even significantly improved when compared to baseline. This again is in line with the findings of preceding controlled clinical trials reporting a beneficial impact of the regular consumption of probiotic strains of *L. reuteri* on parameters of oral health like PPD, gingival inflammation, BoP, plaque coverage of teeth and the composition of the oral microbiota in study participants as diverse as healthy adults<sup>9</sup> healthy pregnant women,<sup>10</sup> healthy infants,<sup>29</sup> periodontitis patients<sup>12,30-34</sup> and type II diabetics.<sup>11</sup>

As already mentioned, the interference of probiotic *L. reuteri* strains with competing bacteria as well as the immune response of their host is very complex. Thus it may not be possible to deduct from the clinical data of this trial whether the marked difference in periodontal health between both experimental groups at the end of the study may be primarily attributed to a direct inhibitory impact of the consumed *L. reuteri* strains on the growth and the metabolism of competing inflammophilic periodontopathogens or may be rather based on a *L. reuteri*-induced downregulation of an excessive proinflammatory host response towards them. Data from other clinical trials reporting a significant decrease in the prevalence of periodontopathogens<sup>14</sup> as well as a significant decrease of proinflammatory cytokines in the sulcular fluid<sup>9</sup> after repeated consumption of probiotic *L. reuteri* strains suggest that both mechanisms may have been involved.

Although in the majority of these studies the consumption of probiotic *L. reuteri*-strains was used as an adjunct to improve the outcome of professional mechanical plaque removal and/or oral hygiene instructions,<sup>9,29</sup> it is important to note, that in the present trial and in a preceding RCT published by the authors<sup>10</sup> the *L. reuteri*-stimulated recovery of oral health parameters was observed in the intentional absence of any concomitant professional mechanical plaque control measures or oral hygiene instructions. The magnitude of the observed improvement of the primary and all secondary study outcomes in the test group and their concomitant deterioration in the placebo group clearly prove that the regular consumption of the evaluated *L. reuteri*-containing lozenges alone without any further attempt to improve the inadequate (baseline PCR  $> 80\%$ ) personal oral hygiene of the study participants was sufficient to recover and stabilize

**TABLE 3** Gingival index and plaque control record (PCR) scores (intent to treat LOCF analysis)

Visit	Placebo group <i>n</i> = 35	Test group <i>n</i> = 33	Difference between groups
<b>Gingival index</b>			
Baseline mean $\pm$ SD	1.3 $\pm$ 0.4	1.3 $\pm$ 0.5	
Day 14 mean $\pm$ SD	1.3 $\pm$ 0.4	0.7 $\pm$ 0.4	
Difference baseline-day 14 mean (95% CI)	-0.1 (-0.2 to -0.0)	-0.6 (-0.7 to -0.1)	-0.5 (-0.6 to -0.4)
<i>P</i> between groups			<0.001
<i>P</i> within groups	0.03	<0.001	
Day 42 mean $\pm$ SD	1.5 $\pm$ 0.4	0.5 $\pm$ 0.4	
Difference baseline-day 42 mean (95% CI)	+0.1 (0.0 to +0.2)	-0.8 (-0.9 to -0.6)	-0.9 (-1.1 to -0.8)
<i>P</i> between groups			<0.001
<i>P</i> within groups	0.01	<0.001	
<b>Plaque control record %</b>			
Baseline mean $\pm$ SD	88 $\pm$ 11	83 $\pm$ 11	
Day 14 mean $\pm$ SD	90 $\pm$ 11	74 $\pm$ 11	
Difference baseline-day 14 mean (95% CI)	+2 (-2 to +6)	-8 (-14 to -3)	-16 (-22 to 10)
<i>P</i> between groups			<0.001
<i>P</i> within groups	0.35	0.004	
Day 42 mean $\pm$ SD	94 $\pm$ 10	75 $\pm$ 15	
Difference baseline-day 42 mean (95% CI)	+5 (+2 to +9)	-7 (-13 to -2)	-15 (-21 to -10)
<i>P</i> between groups			<0.001
<i>P</i> within groups	0.003	0.006	

Comparisons between the groups: Analysis of covariance.

Comparisons between the groups: Wilcoxon signed-rank test.

**TABLE 4** Probing pocket depth (PPD) and probing attachment level (intent to treat LOCF analysis)

Visit	Placebo group <i>n</i> = 35	Test group <i>n</i> = 33	Difference between groups
<b>Probing pocket depth (PPD)</b>			
Baseline mean $\pm$ SD	1.7 $\pm$ 0.4	1.7 $\pm$ 0.6	
Day 14 mean $\pm$ SD	1.7 $\pm$ 0.5	1.6 $\pm$ 0.4	
Difference baseline-day 14 mean (95% CI)	+0.0 (-0.0 to +0.1)	-0.2 (-0.2 to -0.1)	-0.2 (-0.3 to -0.1)
<i>P</i> between groups			<0.001
<i>P</i> within groups	0.92	<0.001	
Day 42 mean $\pm$ SD	2.0 $\pm$ 0.5	1.5 $\pm$ 0.4	
Difference baseline-day 42 mean (95% CI)	+0.2 (+0.1 to +0.3)	-0.3 (-0.4 to -0.1)	-0.5 (-0.6 to -0.4)
<i>P</i> between groups			<0.001
<i>P</i> within groups	<0.001	<0.001	
<b>Probing attachment level (PAL)</b>			
Baseline mean $\pm$ SD	1.8 $\pm$ 0.5	1.8 $\pm$ 0.6	
Day 14 mean $\pm$ SD	1.8 $\pm$ 0.5	1.7 $\pm$ 0.4	
Difference baseline-day 14 mean (95% CI)	+0.1 (-0.0 to +0.1)	-0.2 (-0.3 to -0.1)	-0.3 (-0.3 to -0.1)
<i>P</i> between groups			<0.001
<i>P</i> within groups	0.23	<0.001	
Day 42 mean $\pm$ SD	2.1 $\pm$ 0.5	1.6 $\pm$ 0.4	
Difference baseline-day 42 mean (95% CI)	+0.3 (+0.2 to +0.4)	-0.2 (-0.4 to -0.1)	-0.5 (-0.7 to -0.4)
<i>P</i> between groups			<0.001
<i>P</i> within groups	<0.001	<0.001	

Comparisons between the groups: Analysis of covariance.

Comparisons between the groups: Wilcoxon signed-rank test.





**TABLE 5** Probing pocket depth (PPD) and probing attachment level (PAL) separated for participants with a baseline diagnosis of gingivitis (PSR = 2) and periodontitis (PSR ≥ 3)

Visit	Gingivitis (PSR = 2)			Periodontitis (PSR ≥ 3)		
	Placebo group <i>n</i> = 18	Test group <i>n</i> = 15	<i>P</i> between the groups	Placebo group <i>n</i> = 17	Test group <i>n</i> = 18	<i>P</i> between the groups
<b>Probing pocket depth (PPD)</b>						
Baseline mean ± SD	1.41 ± 0.15	1.50 ± 0.23	0.47	2.10 ± 0.40	1.95 ± 0.70	0.18
Day 14 mean ± SD	1.49 ± 0.24	1.36 ± 0.18	0.08	1.97 ± 0.53	1.71 ± 0.48	0.10
Day 42 mean ± SD	1.69 ± 0.26	1.34 ± 0.18	<0.001	2.24 ± 0.52	1.62 ± 0.44	0.002
<i>P</i> within the groups Baseline-day 14	0.12	0.01		0.13	<0.001	
<i>P</i> within the groups Baseline-day 42	<0.001	0.003		0.03	0.003	
<b>Probing attachment level (PAL)</b>						
Baseline mean ± SD	1.44 ± 0.17	1.56 ± 0.23	0.25	2.20 ± 0.41	2.10 ± 0.64	0.16
Day 14 mean ± SD	1.59 ± 0.53	1.47 ± 0.20	0.14	2.12 ± 0.53	1.90 ± 0.49	0.14
Day 42 mean ± SD	1.79 ± 0.28	1.42 ± 0.18	<0.001	2.43 ± 0.51	1.74 ± 0.42	<0.001
<i>P</i> within the groups Baseline-day 14	0.02	0.08		0.28	0.002	
<i>P</i> within the groups Baseline-day 42	<0.001	0.02		0.01	0.004	

Comparisons within the groups: Wilcoxon signed-rank test.

Comparisons between the groups: Mann-Whitney *U* Test.

the assessed markers of periodontal health in the otherwise medically inconspicuous study population. Regular tobacco smoking, a known major health hazard also to the integrity of oral health, was very prevalent among the study participants. 62% of them reported a smoking experience >5 pack-years (see Table 1). It is remarkable therefore that in a subanalysis comparing smokers and non-smokers of the test group the extent of the observed improvement of the primary outcome at day 42 did not differ significantly (data not shown). This is line with findings from other studies evaluating the effect of *L. reuteri* as an adjunct to non-surgical periodontal treatment of chronic periodontitis in smoking patients.<sup>35</sup> In clinical trials the possible interference of undocumented parameters with the study results is always a potential threat to the validity of the data and may never be completely avoided. The performance of this clinical trial in the confinements of a naval ship at sea provided the rare opportunity to standardize more potentially interfering background factors than usually possible. All included study participants had to strictly follow standardized fixed duty rosters with 8-hours or 24-hours working shifts. The variety of leisure time activities on board was limited and also the daily diet was mostly confined to the variety of meals offered by the galley. The evaluation of the sugar consumption questionnaire did not reveal any significant differences between the groups during the observation period. However total daily sugar intake of all study participants from main meals and sweet snacks clearly exceeded current WHO recommendations (25 g/d) (data not shown). Differences between both experimental groups regarding the type of working shift were also not significant (data not shown). Further the status of good general health and good physical

fitness of the study population had been verified by a medical examination before boarding the ship.

As mentioned before, the evaluation of the study outcomes had to be restricted to the Ramfjord teeth because of the limited availability of evaluation time. This may have led to an underestimation of the true extent of gingival inflammation within the dentition. Nevertheless the general validity of measurements limited to the evaluation of the Ramfjord teeth has been verified by previous studies.<sup>24,36,37</sup> Further, any possible systematic bias introduced by an evaluation restricted to the Ramfjord teeth, would affect both experimental groups equally.

## 5 | CONCLUSION

The regular consumption of lozenges containing the probiotic *L. reuteri*-strains DSM 17938 and ATCC PTA 5289 is an efficacious and easily implementable measure to maintain or improve periodontal health in medically healthy persons independent of the efficacy of personal oral hygiene. This may offer new therapeutic and preventive options in the clinical control of plaque-related gingival inflammations in individuals with impaired access to efficacious oral hygiene.

## CONFLICT OF INTEREST AND SOURCES OF FUNDING STATEMENT

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supplied the experimental lozenges free of charge, but was not involved in data recording, data analysis, or the interpretation of the data. BioGaia AB received the manuscript for approval before submission, but did not interfere with wording, content or emphasis. U. Schlagenhauf reports lecturing fees from BioGaia AB outside the submitted work. All other authors declare that they do not have a conflict of interest. Dr. Juliane Rehder is working for the German armed forces, but the manuscript is not submitted as an official statement or on behalf of the German armed forces.

## AUTHOR CONTRIBUTIONS

US designed the study and prepared the manuscript. JR did all the clinical examinations. GG performed the statistical analysis of the data. YJ-S designed the study and prepared the manuscript. Ulrich Schlagenhauf and Juliane Rehder contributed equally to the work.

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