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Impact of a 7-day field training on oral health condition in Japan Ground Self-Defense Force personnel

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INTRODUCTION

In the Japan Ground Self-Defense Force (JGSDF), personnel periodically perform intensive training that mimics the conditions seen in battle and during natural disasters. The training programs for military personnel may be very strict, involving not only long-term heavy physical activity but also exposure to psychological stressors, sleep deprivation, shifts in circadian rhythm, and exposure to extreme hot and cold environments.[1] Such challenges have an impact on a participant's health condition. Many reports have demonstrated the impacts of stressful conditions on the immune responses of human subjects.[2-4] Military training involves intensive stressful conditions; thus, decreased immunoglobulin A[5] and increased levels of interleukin-6[6], toll-like receptor 4, and tumor necrosis factor-α[7] have been found in personnel following training. Military personnel have difficulty in performing daily oral care to maintain good oral condition under the conditions associated with training.[8] Škec[9] examined oral hygiene in army recruits and emphasized regular checkups for combat readiness. Furthermore, the incidence of severe oral conditions, including acute necrotizing ulcerative gingivitis, has been reported during rescue activities[10]. Good

oral condition is important for the personnel to fulfill their duties. To our knowledge, no studies have examined the effects of a 7-day physically and psychologically stressful training on the oral condition of military personnel by comparing participants' oral condition before and just after training. More than 700 species of bacteria are present in the oral cavity as biofilm constituents, competing with each other in their own community. The pathogenicity of these oral bacteria contributes to infectious diseases, including dental caries and periodontal diseases in the oral cavity. In addition, many investigators have shown that oral bacteria are associated with general health impairment, including cardiovascular diseases, pneumonia, and abscess formation.[11-13] The human immune system defends against bacterial attack to maintain healthy body function. However, conditions of excessive physical or psychological stress can result in dysfunction of these mechanisms of homeostasis. The oral cavity is at risk of damage by stressful conditions due to persistent bacterial attack. This study was performed to clarify the impact of a 7-day training on oral health status among JGSDF personnel. Oral health behaviors before training and during the training period were examined using a self-administered questionnaire.

We examined periodontal condition and determined bacterial contents in dental plaque samples, antimicrobial factors, and a stress marker in saliva samples before and just after the 7-day training period. Changes in bacterial content as well as antimicrobial factors and stress markers during the training period provide useful information for developing new strategies for maintaining healthy oral function in personnel. The presence of eight species of bacteria in dental plaque, including commensal streptococci that are early colonizers on the tooth surface, cariogenic bacteria, and periodontopathic bacteria, were determined using real-time polymerase chain reaction. These data were used to examine the impact of changes in oral health behavior on periodontal condition and changes in oral bacterial content and salivary components.

MATERIALS AND METHODS

Participants

Fifty-nine male and three female JGSDF personnel undergoing 7-day field training in Japan, participated in this study. In field training, personnel continuously act under the conditions of a disaster, an emergency event, or combat, day and night, over a long

period. The subjects ranged in age from 20 to 53 years (34.7 ± 8.8 years, mean ± SD). All personnel were in good general health before training, and no significant impairments occurred in their health condition during the training period. All personnel provided informed written consent to participation after receiving sufficient explanation of the study. We instructed the personnel to perform oral hygiene practices in the same way as they did during previous training periods. This study was approved by the ethics committee of the Kagoshima University Graduate School of Medical and Dental Sciences (No. 284, 446, 557).

Questionnaires for oral health behavior

After training had finished, the personnel were asked to complete a self-administered questionnaire about oral health behaviors before training and during the training period. The questionnaire assessed the frequency of tooth brushing, mouthwash rinsing, gum chewing, and snacking.

Oral examination

Dental caries and periodontal status were examined to evaluate oral health status. Dental caries status, including decayed, missing and filled teeth (DMFT)[14] was evaluated before training. Periodontal status was evaluated before and immediately after training using the community periodontal index (CPI) according to WHO criteria[15]. Briefly, an examiner assessed three indicators of periodontal status using WHO CPI probes: gingival bleeding, calculus, and periodontal pockets for 10 index teeth, categorized in sextants. Each sextant was assigned a code number that indicated the condition of most seriously affected site in that sextant according to the following criteria: code 0 = healthy; code 1 = bleeding observed after probing the gingival sulcus; code 2 = calculus detected during probing; code 3 = periodontal pocket (4-5 mm); code 4 = periodontal pocket (6 mm or deeper). The highest code across sextants for each subject was defined as the "individual CPI code". A single dentist (K.Y.) performed oral examinations throughout the survey, thus eliminating inter-examiner variability.

Sample collection

Plaque and saliva samples were collected before and immediately after training.

Supragingival plaque samples were collected from sound buccal surfaces of maxillary first molars using sterile dental explorers. Samples were suspended in 0.5 mL of sterile phosphate buffered saline (PBS, pH 7.0) and stored at -30°C until further analysis. Stimulated whole saliva was collected by chewing a piece of gum with no taste. After chewing the gum and swallowing saliva for the first 30 s, participants expectorated whole saliva into a tube on ice for 2 min. Samples were then stored at -30°C until further analysis.

Bacterial quantification using real-time polymerase chain reaction

Chromosomal DNA was extracted from collected plaque samples according to a method described previously.[16] Briefly, collected plaque suspension was centrifuged, and the resultant precipitates were resuspended in 100 μL of lysis buffer (20 mM Tris/HCl, 2 mM EDTA, 1% Triton X-100; pH 8.0). The lysate was boiled at 100°C for 10 minutes, and chromosomal DNA was obtained by centrifugation.

The primers used for the quantification of the bacteria are shown in Table I. For real-time PCR, 20 μL of a mixture containing 3 μL of lysed sample, 10 pmol of each

primer, and 10 μL of Fast SYBR Green Master Mix (Thermo Fisher Scientific, Waltham, Massachusetts) was placed in each well of a 48-well plate. The samples were amplified using the StepOne™ Real-time PCR System (Thermo Fisher Scientific). Amplification consisted of an initial denaturation step at 95°C for 20 seconds, followed by 45 cycles at 95°C for 3 seconds and 60°C for 30 seconds. The percentages of specific bacteria were calculated based on the comparative *Ct* (ΔΔ*Ct*) method using the following equation: $\% = 1/2^{(Ct\ \text{target} - Ct\ \text{total})} \times 100$.[16]

Measurement of salivary components

Lactoferrin

Sandwich ELISA was performed to determine lactoferrin concentration following the method of Franco *et al*.[17] Maxisorp micro-titration plates (Nunc, Roskilde, Denmark) were coated with 100 μL per well of goat anti-human lactoferrin antibody (1 μg/mL, Betyl Laboratories Inc., Montgomery, Texas) in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6) and incubated overnight at 4°C. After the plates were washed three times with 300 μL per well of PBS containing 0.05% Tween 20 (PBST, pH 7.4),

residual protein binding sites were blocked with 100 µL of 1% (wt/vol) BSA in PBST at 37°C for 1.5 h. The wells were washed with PBST, and samples and human milk lactoferrin standards (AbD Serotec, Hercules, California) from 1.56 to 50 ng/mL were added to the wells (100 µL each) and incubated for 1.5 h. The plates were then washed with PBST and incubated with 100 µL of HRP-conjugated goat anti-human lactoferrin antibody (Betyl Laboratories Inc.) diluted 1:10000 in PBST for 1.5 h at 37°C. Then, the plates were washed with PBST and incubated with 100 µL of ABTS substrate solution per well for 60 minutes at 37°C. The absorbance at 405 nm was determined using a microplate reader (Model 680, Bio-Rad Laboratories, Hercules, California). The lactoferrin concentration was determined using the standard curve, and interpolation was performed ($r^2 = 0.99$).

LL37

Sandwich ELISA was performed for measurement of LL37. Briefly, micro-titration plates were coated with anti-LL37 IgG antibody[18] (10 µg/mL) in coating buffer. After blocking, samples and human LL37 standards[19] from 62.5 to 1000 ng/mL were added to

the wells. LL37 was detected using biotinylated anti-LL37 IgG antibody (0.9 µg/mL), followed by reaction with alkaline phosphatase-conjugated streptavidin (Vector Laboratories, Burlingame, California). Color development was performed using *p*-nitrophenyl-phosphate in diethanolamine buffer, and absorbance at 405 nm was determined. The LL37 concentration was determined using the standard curve.

Lysozyme

Lysozyme concentration was determined by dot blot assay. Samples and human recombinant lysozyme standards (Wako Pure Chemical Industries Ltd., Osaka, Japan) from 31.2 to 2000 ng/mL in 100 µL of Tris buffered saline (TBS; 20 mM Tris, 0.15 M NaCl, pH 7.2) were blotted onto nitrocellulose membranes using a Bio-Dot microfiltration apparatus (Bio-Rad Laboratories). The membranes were blocked with 3% skimmed milk in TBS containing 0.1% Triton-X 100 (TBST) for 1.5 h at room temperature. After washing with TBST, the membranes were incubated with rabbit anti-human lysozyme antibody (Nordic Immunological Laboratory, Eindhoven, The Netherlands) diluted 1:1000 in TBST for 1.5 h at room temperature. The membranes

were washed with TBST and incubated with alkaline phosphatase-conjugated goat anti-rabbit IgG (Zymed Laboratories, San Francisco, California) in TBST for 1.5 h at room temperature. Colorimetric development was performed using a BCIP/NBT tablet (Sigma Chemical Co., St. Louis, Missouri), and the lysozyme concentration was quantified using Image J software (http://imagej.nih.gov/ij/).

α-amylase

The intensity of physical or psychological stress was monitored by measuring the level of α-amylase activity using a commercial kit (α-Amylase Measuring Kit; Kikkoman, Tokyo, Japan). In the assay, α-amylase in the saliva sample reacts with the substrate, yielding 2-chloro-4-nitrophenol, which is yellow in color and can be detected using a spectrophotometer at 405 nm.

Statistical analysis

The differences between groups were analyzed using Student's *t*-test, Wilcoxon signed-rank test, Mann-Whitney *U* test, or chi-square test as appropriate. Logistic

regression analysis was performed, with CPI change after training as the dependent variable. We compared the CPI code in each sextant for each subject before with that after training, and the number of sextants exhibiting an increase in CPI code was calculated. The participants were divided into two groups according to the number of CPI sextants that deteriorated (no/slight deterioration = 0-2, severe deterioration = 3-5). The independent variables were behavior frequencies during the training period, which were categorized as follows; tooth brushing (0, ≥1), mouthwash rinsing (0, ≥1), gum chewing (0, ≥1), and snacking (≥1 time per 2 days, <1 time per 2 days). Multiple regression analysis was performed to gauge the effect of each health behavior on the bacterial percentage. All statistical analyses were performed using SPSS version 20 (IBM SPSS Japan, Tokyo, Japan). In all analyses, $P < 0.05$ was taken to indicate statistical significance.

RESULTS

Oral health behavior and oral health status

The frequencies of oral health behaviors before and during the training period were

compared. All personnel performed tooth brushing every day before training, but the percentage decreased to 6.5% during training, and 40.3% of personnel did not brush their teeth at all during the training period. The number of personnel who snacked every day or every 2 days increased markedly during the training period. There were significant differences between the frequencies of tooth brushing and snacking before and those after training ($P < 0.05$, Wilcoxon signed-rank test).

Periodontal condition was examined using CPI. Changes in individual CPI code between before and after training are shown in Table II. Thirty-five personnel (56.5%) showed an increase in individual CPI code after training; twenty-four from code 0, six from code 1, four from code 2, and one from code 3. A significant difference in code distribution between before and after training was observed ($P < 0.05$, Wilcoxon signed-rank test). Next, we counted the number of CPI sextants that deteriorated after training in each subject (Figure 1). Fifty-seven individuals (91.9%) showed deterioration of the CPI code in one or more sextants after training, and 16 (25.8%) showed deterioration in two sextants.

The effects of oral health behaviors during the training period on periodontal

condition were examined. We divided personnel into two groups (no/slight deterioration, severe deterioration) according to the number of CPI sextants exhibiting deterioration. The relationship between oral health behavior and CPI deterioration is shown in Table III. There was a significant difference in tooth brushing frequency between the two groups. However, there were no differences in the frequency of mouthwash rinsing, gum chewing, or snacking between the two groups. Logistic regression analysis indicated that only tooth brushing frequency was significantly associated with CPI deterioration; the odds ratio in personnel who did not brush their teeth was 7.51 relative to those who did brush at least once during the training period (*P*-value, 0.002; 95% CI, 2.04-27.65).

Effects of dental caries experience on periodontal condition and oral health behavior

We examined the association of dental caries experience with the change in periodontal condition during training. The number of DMF teeth (DMFT) of all personnel examined was 10.5 ± 6.1 (mean ± SD); subjects were divided into two groups according to the mean value of DMFT: 5.3 ± 3.5 for the low-DMFT group (n = 30) and 15.4 ± 3.3

for the high-DMFT group (n = 32). The distributions for all groups according to the number of CPI sextants exhibiting deterioration after training are shown in Figure 2. The personnel in the low-DMFT group mainly showed slight deterioration in CPI, whereas the personnel in the high-DMFT group showed severe deterioration in CPI. The difference in distributions of the number of CPI sextants exhibiting deterioration between the two groups was significant. None of the personnel in the high-DMFT group brushed their teeth every day (Table IV). The percentage of personnel who did not perform tooth brushing during the training period in the high-DMFT group was approximately double that in the low-DMFT group. The difference in tooth brushing frequency between the two groups was significant.

Relationship between oral health behaviors and bacterial content in dental plaque

The percentages of bacteria in dental plaque before and after training are shown in Table V. The percentages of *Streptococcus sanguinis* and *Streptococcus gordonii* increased significantly, whereas those of *Streptococcus mutans*, *Streptococcus sobrinus*, and *Aggregatibacter actinomycetemcomitans* decreased significantly following training.

We examined the association of oral health behaviors during the training period with the change in plaque bacterial content. Personnel who did not brush their teeth or rinse with mouthwash showed significant increases in the *S. sanguinis* percentage (Table VI). Multiple regression analysis indicated that tooth brushing frequency was significantly associated with *S. sanguinis* percentage after training (standardized partial regression coefficient β-weight = 0.281, $P = 0.041$).

Salivary components

Salivary contents of lactoferrin, LL37, and lysozyme were measured to examine the effects of training. Lactoferrin concentration (mean ± SD μg/mL) increased significantly after training (22.5 ± 27.6) compared with baseline (16.7 ± 19.4), whereas LL37 and lysozyme showed no changes ($P < 0.05$, Wilcoxon signed-rank test). α-amylase activity was measured to examine stress levels induced by training, but no significant changes were observed after training.

DISCUSSION

The present study was performed to examine the impact of changes in oral health behavior on periodontal condition and changes in oral bacterial contents and salivary components among JGSDF personnel after a 7-day training. Before training, all of the JGSDF personnel brushed their teeth every day, and 41.9% showed no gingival bleeding (CPI = 0). With regard to the daily oral hygiene routine (i.e., before training) and oral health status, Škec[9] reported that 97.4% of Croatian recruits had habitually brushed their teeth every day, and approximately 36% showed no gingival bleeding. Senna *et al*.[20] also reported that 40.95% of Italian call-up soldiers had healthy periodontal condition. The tooth brushing frequency and periodontal condition of the JGSDF personnel before training in the present study were compatible with these previous reports. The frequencies of tooth brushing and snacking changed during the training period. Tooth brushing frequency decreased during the training period, and similar results were reported in JGSDF personnel.[8] Personnel were engaged in persistent field training, and it seemed to be difficult to perform tooth brushing regularly. The frequency of snacking increased during the training period. Personnel had to take meals at irregular times during the training period, and they may have tried to

supplement their energy by frequent snacking. These changes in behavior seemed to result in impairment of oral condition. In fact, the periodontal condition of participants deteriorated during the training period, and the distribution of individual CPI codes shifted, indicating deterioration. To elucidate the cause of periodontal deterioration, the relationship between oral health behaviors and periodontal condition was examined. Twenty-three of 37 personnel who brushed their teeth at least once during the training period showed no/slight deterioration in CPI, whereas 19 of 25 personnel who did not brush during the training period showed severe deterioration. Logistic regression analysis revealed a significant association between CPI deterioration and tooth brushing frequency (odds ratio = 7.51). Löe *et al*.[21] reported that the Gingival Index increased in healthy persons after 7 days of interrupted oral hygiene. Our results are consistent with their results and indicate that tooth brushing is an important behavior to prevent periodontal deterioration during the training period. Regarding the change in periodontal condition after a 7-day training, major changes were observed in the gingiva, but no change was observed in calculus deposition. The use of the Gingival Index [22] may have more accurately measured gingival changes seen after 7 days of poor or no

oral hygiene.

To examine the effects of dental plaque bacteria on gingival condition, changes in bacterial content during the training period were examined. Dental plaque is composed of many bacterial species, and the microbial interactions in the communities have been investigated.[23-25] Dental plaque formation is initiated by the attachment of early colonizers, including *S. sanguinis*, *Streptococcus oralis*, and *S. gordonii*, on salivary pellicle-coated enamel surfaces.[26] Late colonizers, including periodontopathic bacteria such as *Porphyromonas gingivalis*, *A. actinomycetemcomitans*, and *Treponema denticola*, bind to previously bound bacteria, and sequential binding occurs by bridging with the next coaggregating partner cells.[23] Between early and late colonizers, *Fusobacterium nucleatum*, the most numerous Gram-negative species in healthy sites, acts as a "bridge" by coaggregating with both types of colonizer.[27] Cariogenic streptococci, including *S. mutans* and *S. sobrinus*, are not abundant in the initial colonizing community on the tooth surface. If sucrose, as a carbohydrate substrate, is introduced into the community, the bacteria produce exopolysaccharides, such as water-insoluble glucan, which act to accumulate many species forming a cariogenic

biofilm.[28] Therefore, we selected eight specific bacteria described in Table I. The percentages of two species, *S. sanguinis* and *S. gordonii*, increased after training. Socransky *et al*.[29] examined bacterial species present at various times during plaque development on the tooth surface and demonstrated that *S. sanguinis* was consistently present, becoming predominant at days 1-2 and thereafter. Takeshita *et al*.[30] examined the assembly process of dental plaque microbiota on hydroxyapatite disks in young adults and found an increase in the relative abundance of *S. gordonii* on day 7 compared to that on day 1. Our results are consistent with these previous studies. *S. sanguinis* and *S. gordonii* are known to produce hydrogen peroxide as an antibacterial agent against other bacterial species.[31, 32] The increase in these two streptococci seems to be due to their high capacity to produce hydrogen peroxide. On the other hand, the percentage of *S. mutans*, *S. sobrinus*, and *A. actinomycetemcomitans* decreased significantly after training. These bacteria may have been affected by interaction among antimicrobial agents released by the dominant bacteria. It is also possible that the percentages of these bacteria decreased relative to the increments in other dominant bacteria. Regarding the effects of oral health behaviors, an increase in *S. sanguinis*

percentage was observed after discontinuation of tooth brushing or mouthwash rinsing, and tooth brushing frequency was significantly associated with *S. sanguinis* percentage after training. Taken together, the results indicate that plaque maturation occurred following discontinuation of tooth brushing and induced periodontal inflammation during the training period.

Among the salivary components, the concentration of lactoferrin, an ion-binding protein found in secreted fluids including saliva, milk, tears, etc.,[33] increased after training. Lactoferrin is released by neutrophil activation, and the molecule has been investigated in studies on inflammatory diseases.[34, 35] With regard to periodontal disease, increases in lactoferrin concentration in the gingival crevicular fluid (GCF) of gingivitis patients[36] and in the saliva of periodontitis patients[37] have been reported. The increase in lactoferrin level found in the present study seemed to be caused by the inflammatory changes in periodontal tissues during the training period. On the other hand, LL37 and lysozyme concentrations showed no changes after training. LL37 is an antimicrobial peptide and lysozyme is a defense protein, and both antimicrobial agents are present in saliva and GCF.[38, 39] Correlations between these antimicrobial

agents and periodontal diseases have been reported. Surna *et al*. [40] demonstrated an increase in lysozyme concentration in GCF, but not in saliva, of gingivitis and periodontitis patients, findings that are compatible with our results. Regarding the change in LL37 concentration in periodontal disease patients, an increase in GCF has been reported,[41] but no reports have shown a change in saliva. Not only inflammation but also physical stressors have been reported to induce LL37 and lysozyme.[42, 43] To clarify the level of stress, we measured salivary α-amylase activity as a stress marker. In numerous previous studies, salivary α-amylase has been an effective stress marker for the autonomic/sympathetic nervous system;[44] the α-amylase response to a stressor occurs more rapidly than changes in salivary cortisol.[45] The participants showed no changes in the α-amylase level after training. The training intensity may have been insufficient to elevate the α-amylase level, or these personnel may have adapted to such a load through periodic training activity. In any case, the stress level induced by this training may have not been sufficient to elevate LL37 and lysozyme.

Periodontal deterioration during the training period was associated with dental caries experience, and severe periodontal deterioration was observed in the high-DMFT

group. Tooth brushing frequency during the training period decreased more in the high-DMFT than in the low-DMFT group. DMFT, the accumulated number of teeth affected by dental caries, reflects a subject's total caries experience including past and present caries.[14] Both dental caries and periodontal disease result from the prolonged presence of pathogenic plaque bacteria, which affect the teeth and periodontal tissues; these can be controlled by mechanical and chemical plaque control regimens. It is thought that personnel in the high-DMFT group did not have proper plaque control habits in childhood and adolescence, and such habits could have resulted in a decline in tooth brushing frequency during the stressful training period. It is also possible that the high-DMFT group may have had an increased plaque Retention Index.[22] The results suggest that guidance to maintain continuous tooth brushing is necessary for personnel with a high experience of dental caries.

In conclusion, we demonstrated periodontal deterioration in JGSDF personnel after a 7-day training. Behavioral changes, especially discontinuation of regular tooth brushing, fostered dental plaque maturation, resulting in the inflammatory changes seen in participants' periodontal condition and the elevation of salivary lactoferrin. It would

be of interest to compare the present results with measures of oral health status in other professionals who also work under special circumstances, including police officers and fire fighters. Our results indicate the importance of performing tooth brushing at least once during a 7-day training period to prevent periodontal deterioration. We think that periodontal deterioration would increase during deployments of greater than 7 days because anaerobic bacteria would predominate in the accumulated plaque bacterial community leading to severe periodontal deterioration.[46] Therefore, it is necessary to examine the appropriate frequency of tooth brushing for training periods exceeding 7 days. The regimen could be applicable to evacuees from disasters, including earthquakes and tsunamis, because they are under conditions of stress that may limit oral hygiene activity; however, careful consideration of the differences between subjects and conditions would be necessary. Supervising personnel should enforce tooth brushing at least once during a 7-day training to reduce adverse oral conditions during deployment.

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FIGURE LEGENDS

Figure 1. Distribution of the number of CPI sextants exhibiting deterioration after training.

Figure 2. Distribution of low-DMFT and high-DMFT groups according to the number of CPI sextants exhibiting deterioration after training. Open bar, low-DMFT group; closed bar, high-DMFT group. *$P < 0.05$ between two groups, as determined by Mann-Whitney *U* test.

Figure 1

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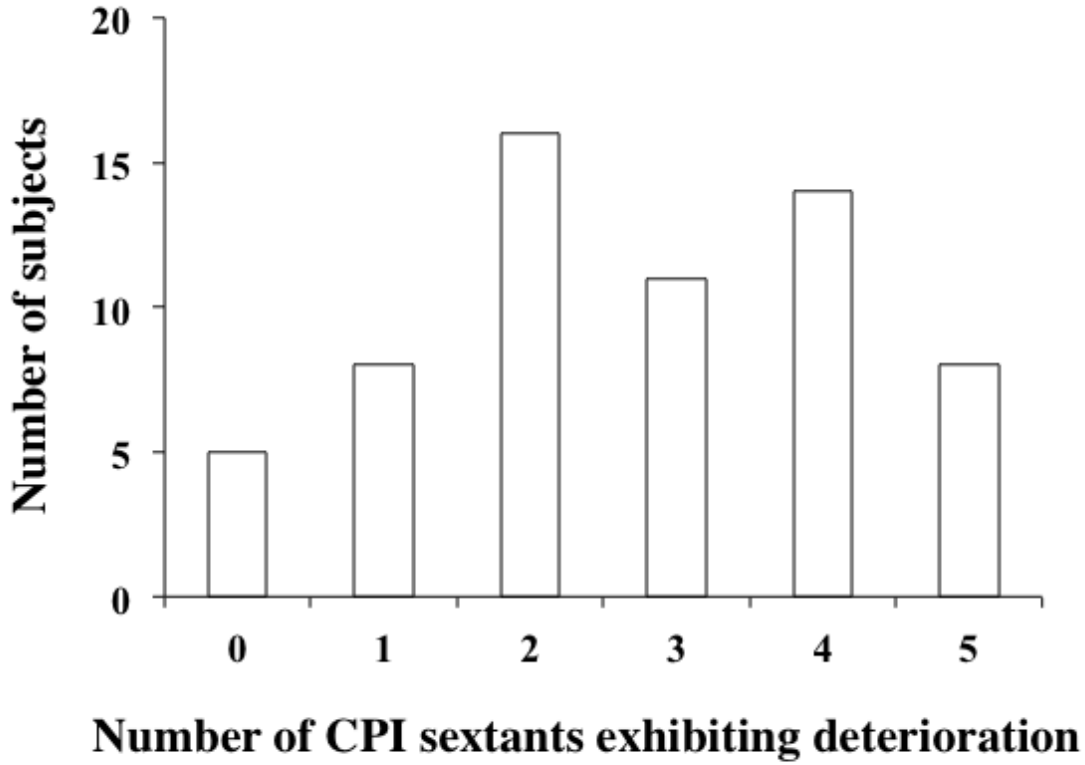

Fig. 1

Figure 2

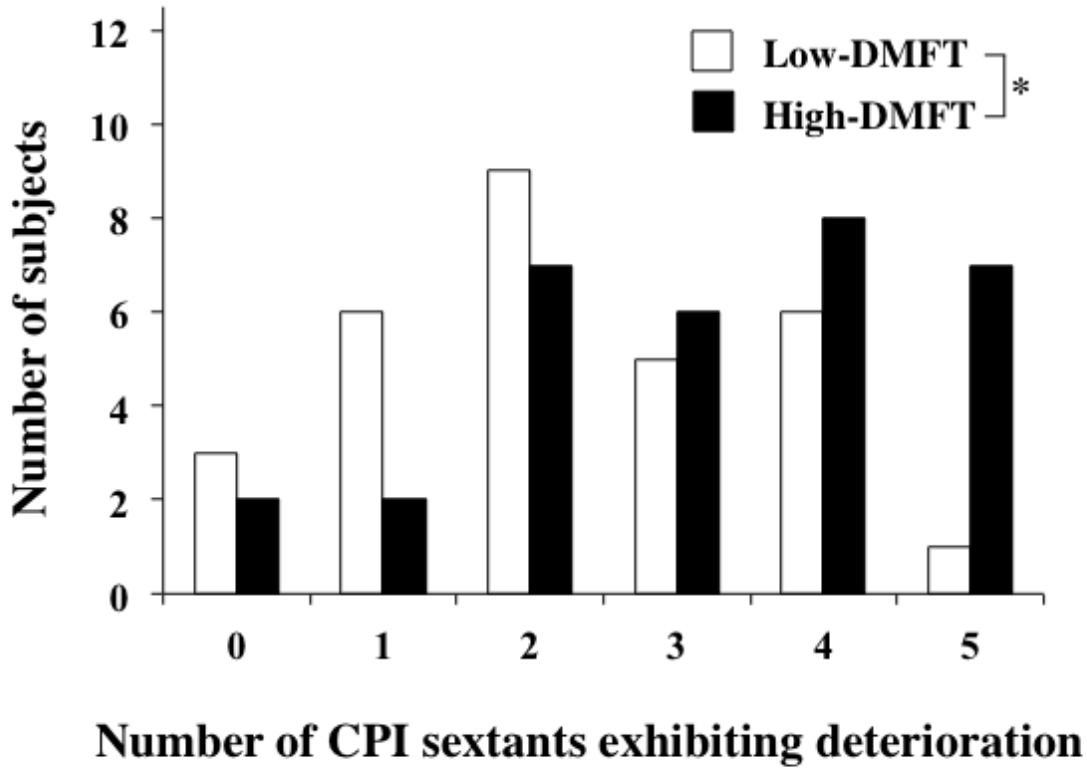

Fig. 2

TABLE I. Oligonucleotide primers

Species	Designation of primers	Sequence	Amplicon size (bp)	Target	Reference
Streptococcus mutans	Smut3368-F	5'-GCCTACAGCTCAGAGATGCTATTCT-3'	114	*gtfB*	16
	Smut3481-R	5'-GCCATACACCACTCATGAATTGA-3'			
Streptococcus sobrinus	Ssob287-F	5'-TTCAAAGCCAAGACCAAGCTAGT-3'	88	*gtfT*	16
	Ssob374-R	5'-CCAGCCTGAGATTCAGCTTGT-3'			
Streptococcus sanguinis	tnpA-F	5'-CAAAATTGTTGCAAATCCAAAGG-3'	74	*tnpA*	47
	tnpA- R	5'-GCTATCGCTCCCTGTCTTTGA-3'			
Streptococcus gordonii	gtfG-F	5'-CGGATGATGCTAATCAAGTGACC-3'	177	*gtfG*	48
	gtfG-R	5'-GTTAGCTGTTGGATTGGTTGCC-3'			

Streptococcus oralis	gtfR-F	5’-ACCAGCAGATACGAAAGAAGCAT-3’	235	*gtfR*	48
	gtfR-R	5’-AGGTTCGGGCAAGCGATCTTTCT-3’			
Porphyromonas gingivalis	Pg1198-F	5’-TACCCATCGTCGCCTTGGT-3’	126	16S rRNA	49
	Pg1323-R	5’-CGGACTAAAACCGCATACACTTG-3’			
A. a.[a]	Aa1956-F	5’-CAGCATCTGCGATCCCTGTA-3’	147	*IktA*	49
	Aa2102-R	5’-TCAGCCCTTTGTCTTTCCTAGGT-3’			
Fusobacterium nucleatum	619-F	5’-CGCAGAAGGTGAAAGTCCTGTAT-3’	101	16S rRNA	50
	719-R	5’-TGGTCCTCACTGATTCACACAGA-3’			
Universal	Uni152-F	5’-CGCTAGTAATCGTGGATCAGAATG-3’	69	16S rRNA	49
	Uni220-R	5’-TGTGACGGGCGGTGTGTA-3’			

[a]*Aggregatibacter actinomycetemcomitans*

TABLE II Change in CPI individual code before and after training

CPI individual code	CPI individual code after training					Total
before training	0	1	2	3	4	
0	2[a]	20	0	4	0	26
1	0	10	0	6	0	16
2	0	0	5	4	0	9
3	0	0	0	10	1	11
Total	2	30	5	24	1	62

[a]Values represent number of personnel.

TABLE III. Relationship between oral health behavior during training period and CPI deterioration

Behavior	CPI deterioration		*P*-value[a]
	No/slight	Severe	
Tooth brushing			0.004
At least once	23[b]	14	
Not doing	6	19	
Mouthwash rinsing			0.696
At least once	4	3	
Not doing	25	30	
Gum chewing			0.798
At least once	11	14	
Not doing	18	19	
Snacking			0.409
Once or more/two days	22	21	
Less than once/two days	7	12	

[a]Determined by chi-square test.

[b]Values represent number of personnel.

Table IV. Relationship between tooth brushing frequency during training period and dental caries experience

Tooth brushing frequency	Low-DMFT	High-DMFT*
Every day	4 (13.3)[a]	0 (0)
Once per two days	6 (20.0)	6 (18.8)
Once per three or four days	9 (30.0)	7 (21.9)
Once per five-seven days	3 (10.0)	2 (6.3)
Not doing	8 (26.7)	17 (53.1)

[a]Values represent number of personnel (%).

*$P < 0.05$ between two groups, as determined by Mann-Whitney *U* test.

TABLE V. Percentage of bacteria in dental plaque before and after training

Bacterium	Before (%)	After (%)
S. mutans	0.016 ± 0.052[a]	0.004 ± 0.021*
S. sobrinus	0.0008 ± 0.0018	0.0002 ± 0.0010*
S. sanguinis	0.056 ± 0.114	0.148 ± 0.258*
S. gordonii	0.189 ± 0.421	0.307 ± 0.556*
S. oralis	0.124 ± 0.245	0.202 ± 0.333
P. gingivalis	0.016 ± 0.083	0.039 ± 0.109
A. actinomycetemcomitans	0.00008 ± 0.00030	0.00002 ± 0.00004*
F. nucleatum	0.151 ± 0.306	0.173 ± 0.236

[a]Values represent mean ± SD.

*$P < 0.05$ between before and after training, as determined by Wilcoxon signed-rank test.

TABLE VI. Effects of oral health behavior during training period on the change of *S. sanguinis* percentage in dental plaque

Behavior	Before (%)	After (%)
Tooth brushing		
At least once	0.052 ± 0.090[a]	0.089 ± 0.137
Not doing	0.062 ± 0.144	0.235 ± 0.359*
Mouthwash rinsing		
At least once	0.037 ± 0.057	0.042 ± 0.049
Not doing	0.059 ± 0.119	0.161 ± 0.271*

[a]Values represent mean ± SD.

*$P < 0.05$ between before and after training, as determined by Wilcoxon signed-rank test.

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ABSTRACT

Introduction

In the Japan Ground Self-Defense Force (JGSDF), personnel periodically perform intensive training that mimics the conditions seen in battle and during natural disasters. Military training involves intensive, stressful conditions, and changes in immune responses have been found in personnel following training. Good oral condition is important for military personnel to fulfill their duties; however, they have difficulty performing daily oral care under training conditions. In this study, we investigated the impact of a 7-day field training on the oral health status of JGSDF personnel by comparing their oral condition before and just after training.

Materials and Methods

The participants were 59 male and 3 female JGSDF personnel undergoing a 7-day field training. All personnel provided informed written consent to participate, and this study was approved by the ethics committee of the Kagoshima University Graduate School of Medical and Dental Sciences. Oral health behaviors before and during the

training period were surveyed using a self-administered questionnaire. Dental caries was assessed before training in terms of decayed, missing and filled teeth (DMFT), and periodontal condition was examined before and immediately after training using the community periodontal index (CPI). The presence of eight species of bacteria in dental plaque, including commensal streptococci that are early colonizers on the tooth surface, cariogenic bacteria, and periodontopathic bacteria, was determined using real-time polymerase chain reaction. We also assessed antibacterial factors and a stress marker in saliva samples. Sample collection was performed before and just after training. In addition to difference analysis between groups, logistic regression analysis was performed to examine the association between each health behavior and periodontal deterioration.

Results

The frequency of tooth brushing decreased, and snacking increased during the training period. Thirty-five personnel (56.5%) showed an increase in individual CPI code (Table II), and 57 personnel (91.9%) showed deterioration in the CPI code in one or

more sextants after training (Figure 1). Tooth brushing frequency was significantly associated with CPI deterioration; the odds ratio in subjects who did not brush their teeth was 7.51 compared to those who brushed at least once during the training period. Severe periodontal deterioration was observed in the high-DMFT group (Figure 2), and tooth brushing frequency during the training period decreased more in this group compared to the low-DMFT group (Table IV). The percentages of *Streptococcus sanguinis* and *Streptococcus gordonii* increased significantly after the training period suggesting dental plaque maturation (Table V), and an increase in *S. sanguinis* was associated with tooth brushing frequency. The lactoferrin concentration in saliva increased significantly after training.

Conclusions

We demonstrated periodontal deterioration in JGSDF personnel after a 7-day training. Behavioral changes, especially discontinuation of regular tooth brushing, fostered dental plaque maturation, resulting in inflammatory changes in participants' periodontal condition. The results indicate the importance of performing tooth brushing at least

once over a 7-day training period for prevention of periodontal deterioration. The regimen could be applicable to evacuees from disasters because they are under conditions of stress that may limit oral hygiene activity.