Effects of Singing on Oral Function, Stress, and Immunity

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Abstract

Background: We previously reported that singing can improve mental health and oral function. Since the results were based on one singing session, further investigation was required. Therefore, we assessed the effects of daily singing on oral and immune/endocrine function in two studies.

Methods: Thirteen subjects (five men, eight women) with a mean age of 51.3 years (standard deviation [SD] = 13.0) and 17 subjects (six men, 11 women) with a mean age of 46.8 years (SD = 8.0) participated in study 1 and study 2, respectively. The effects of singing on oral function, immunity, and stress were determined by performing a swallowing function test, oral cavity examination, blood and saliva tests, and questionnaires before and after the singing period.

Results: Both chewing and swallowing showed significant improvement with singing (study 1), and saliva production also improved significantly with singing (study 2). In study 1, immunological analysis showed a significant increase of T cells, B cells, CD4+ T cells, and CD8+ T cells with singing, and immunity scores increased significantly from week 4 through week 8 compared to before singing. The mood and emotional state of the participants (visual analog scale) improved in both studies, along with reduction of cortisol and adrenaline, which are indicators of psychological stress. Levels of lipid peroxide and 8-hydroxy-2'-deoxyguanosine, markers of oxidative stress, were also reduced in both studies.

Conclusions: Singing effectively promotes oral function, including saliva secretion and muscle strength, and reduces psychological stress. Singing may also improve immune function and reduce oxidative stress.

Introduction

Oral function strongly influences health and quality of life. Feeding relies on oral function, while speech and facial expressions are crucial for communication [1]. Swallowing is linked to complex higher brain functions, and eating is associated with learning and memory [2,3]. Saliva cleans the oral cavity and reduces micobial proliferation [4-6]. Saliva contains various molecules involved in immunity and health maintenance [7-11], but their secretion decreases with aging [12,13]. Impaired chewing and swallowing are associated with aspiration pneumonia. Among the elderly, pneumonia is a frequently fatal and complications such as diffuse aspiration bronchiolitis can further increase mortality [14]. Hence, the oral cavity is important for both health and quality of life.

Reducing psychological stress may prolong the lifespan [15], while stress caused by isolation increases mortality [16]. Stress also negatively impacts immune function. Accordingly, stress reduction methods have been investigated [17-19].

Increased oral muscle strength is associated with greater secretion of saliva [20], and exercising the mimetic muscles can affect emotions [21]. We previously showed that saliva secretion increases after singing a single song and the participants reported emotional improvement [22]. This time, we investigated the effects of daily singing on oral function and health.

Methods

Study design

An open-label study (study 1) and a randomized, single-blind, cross-over study (study 2) were conducted after receiving approval from the Institutional Review Board of Chiyoda Paramedical Care Clinic (Tokyo, Japan). In accordance with the Declaration of Helsinki, the contents and methods of the study and other pertinent information were adequately explained to the participants, who provided written consent. Both studies were approved by the Institutional Review Board of Chiyoda Paramedical Care Clinic (approval numbers: study 1:13011702; study 2:14102102). Both studies were performed according to the Declaration of Helsinki and the subjects gave informed consent to participation.

Participants

Subjects were recruited by using a website. Study 1 was conducted in 13 subjects (five men and eight women with a mean age of 51.3 years; standard deviation [SD] = 13.0 years) and study 2 enrolled 17 subjects (six men and 11 women with a mean age of 46.8 years; SD = 8.0 years). All subjects satisfied the selection criteria and provided written consent to participation. Exclusion criteria were current treatment for respiratory, cardiac, or vascular conditions; regular intake of health foods that could modulate stress or immunity and have an impact on study outcomes; a history of serious heart, lung, kidney, gastrointestinal (including gastrectomy), hematological, endocrine,

or metabolic disease or current treatment for any of these diseases; difficulty attending t hospital; depression, psychological instability, or strong psychogenic reaction; and any other reason that made the subject unsuitable according to the responsible physician. A profile of the participants is shown in Table 1.

Study schedule and procedures

Study 1 was an open investigation. Subjects were assessed at three baseline visits before the singing period, as well as after four and eight weeks of the singing period. Study 2 was performed according to a cross-over design. Subjects were assessed at four baseline visits before intervention, and then after four weeks of singing or not singing. They subsequently switched to the other intervention and were assessed again after a further 4 weeks. During the singing period of both studies, the subjects sang karaoke four times a week for one hour each time (five songs or more). The frequency of singing was set within the range that would not interfere with daily life. Singing in a karaoke box was selected because it is popular in Japan. Subjects sang alone in the karaoke box and they selected the songs themselves. The subjects were instructed to live normally, but to avoid drinking and eating excessively on the day before and the day of each examination, since overindulgence or poor health (e.g., fever or abdominal pain) could influence secretion of saliva. From 9 p.m. on the day preceding each visit, subjects were prohibited from the following activities until after completing the visit: drinking alcohol, consuming food/drink that could influence stress markers in saliva (green tea, coffee, soda, and high-caffeine beverages such as energy drinks), excessive exercise (physical stress can potentially affect salivary stress markers), and use of antihistamines (which inhibit secretion of saliva). On the examination day, subjects were prohibited from smoking until after completion of the examination. Subjects arrived at Chiyoda Paramedical Clinic in the morning and were interviewed by the study doctor. Then the body weight, blood pressure, and pulse rate were measured. Subsequently, examinations were performed in the following order: resting test, Saxon test, gum test, repetitive saliva swallowing test (RSST), measurement of bite force, questionnaire, and collection blood and urine samples.

Measurements

Blood levels of lipid peroxides, cortisol, and catecholamines (adrenaline and noradrenaline) were analyzed. The immunity score, comprised of the immunity grade and T lymphocyte age, was calculated by the Scoring of Immunological Vigor (SIV) method [23, 24]. All measurements and calculations of immunological markers were performed by the Institute of Health and Life Science (Tokyo, Japan). Urine samples were analyzed to measure 8-hydroxy-2'-deoxyguanosine (8-OHdG). Secretion of saliva was assessed by the resting test, Saxon test, and gum test. Oral function and swallowing were examined by the RSST and bite force measurement. To complete the questionnaire, participants selected answers on a visual analog scale (VAS). In addition, the height, weight, blood pressure, and pulse rate were measured.

Statistical analysis

Results are expressed as the mean ± SD, unless otherwise specified. The Wilcoxon signed-rank test was used to compare data obtained at each visit with the baseline values. All analyses were performed with significance set at $p < 0.05$ (two-tailed) using SPSS 20.0 software (IBM Japan Inc., Tokyo, Japan) and SAS 9.2 software (SAS Institute Inc., North Carolina). In study 2, data obtained by the 2×2 crossover design were analyzed by using PROC GLM in the SAS program.

Results

Compliance with singing therapy

A 100% compliance rate was defined as singing on 32 days (study 1) or 16 days (study 2) for one hour per day, with five or more songs in each singing session. Table 2 shows compliance with singing therapy. The compliance rate was 101.7 ± 4.3% in study 1 because two subjects sang on 33 days and one subject sang on 37 days, while the remaining subjects sang on 32 days as scheduled.

Saliva secretion and oral function

The data on oral function and saliva secretion obtained in study 1 are listed in Table 3. Compared to baseline, the RSST value increased

Table 1. Profile of the participants.

	Study 1			Study 2		
No.	13			17		
Sex (Male/Female)	5	/	8	6	/	11
Age (years)	51.3	±	13	46.8	±	8
Height (cm)	161.06	±	7.86	160.7	±	7
Weight (kg)	61.21	±	15.57	63.28	±	16.56
BMI (kg/m²)	23.552	±	5.691	24.331	±	5.442
SBP (mmHg)	115.7	±	17	115.9	±	14.9
DBP (mmHg)	73.8	±	12.9	73.4	±	11.6
Pulse rate (bpm)	76.5	±	5.4	76.2	±	8.0

Data are shown as the mean± SD
BMI, body mass index; bpm, beats per minute; DBP, diastolic blood pressure; SBP, systolic blood pressure; SD, standard deviation

Table 2. Compliance with Singing Therapy.

	Study 1			Study 2		
No. of days performed	32.5	±	1.4	15.9	±	0.2
Compliance rate (%)*	101.7	±	4.3	99.6	±	1.5
No. of songs per session	10.1	±	2.5	7.8	±	1.4

Data are shown as the mean± SD (n=13)
*100% compliance rate = singing on 4 days per week during the study period.
SD, standard deviation

Table 3. Swallowing and oral function in study 1.

		Baseline			week 4			week 8		
RSST	(times)	4.5	±	1.4	5.4	±	1.3*	5.8	±	1.5**
Bite force test	(kN)	0.087	±	0.082	0.088	±	0.04	0.101	±	0.043*
Unstimulated saliva test	(mL)	3.1	±	3.1	3.3	±	2.9	3.1	±	2.4
Saxon test	(g)	4	±	1.9	4.8	±	1.6	4.8	±	2
gum test	(mL)	15.7	±	8.6	18.1	±	6.1	17.6	±	5.6

Data are shown as the mean± SD (n=13).
*p<0.05, ** p<0.01: Wilcoxon signed-rank test vs. Baseline.
RSST, repetitive saliva swallowing test; SD, standard deviation

significantly during the singing period. Before singing therapy, RSST was 4.5 ± 1.4 per session. After four weeks of singing therapy, RSST increased to 5.4 ± 1.3 per session and it increased further to 5.8 ± 1.5 per session after 8 weeks. In addition, bite force increased significantly from 0.087 ± 0.082 kilonewtons (kN) at baseline to 0.101 ± 0.043 kN in week 8 of the singing period (p=0.028). The gum test and Saxon test values were also increased in both week 4 and week 8 compared with baseline, but the changes were not significant.

Data on oral function and saliva secretion obtained in study 2 are displayed in Table 4. According to the unstimulated saliva test, production of saliva was significantly greater in the singing group compared to the non-singing group during the singing period (p=0.013). In week 4, the RSST, gum test, and Saxon test values were also higher in the singing group compared with the non-singing group, but these differences were not significant.

Immune function

Table 5 lists the results of the immunological tests performed in study 1. The immunity score was significantly increased in week 4 and week 8 compared with baseline, while the immunity grade was significantly increased in week 4 of the singing period. Both CD3+ T cells and CD8+/CD28+ T cells also showed a significant increase in week 8, while CD20+ B cells and naïve T cells were significantly increased in week 4. In weeks 4 and 8, the number of CD4+ naïve T cells and the naïve T cell/ memory T cell ratio showed a significant increase. Finally, the CD4+ memory T cell count was significantly decreased in weeks 4 and 8, while CD56–/16+ NK cells were significantly decreased in week 8.

Hormones and oxidative parameters

Table 6 shows the results for study 1. Compared with baseline, the blood level of lipid peroxide was significantly decreased in week 4 of the singing period. In addition, blood levels of cortisol and adrenaline were significantly decreased in week 8. While the urine level of 8-OHdG was decreased in weeks 4 and 8, the change was not significant.

The results for study 2 are shown in Table 7. Compared with the non-singing group, urine 8-OHdG in was decreased at 4 week in the singing group, but the change was not significant.

Questionnaire

Results of the questionnaire are shown in Table 8 (study 1) and Table 9 (study 2). In study 1, there was significant improvement of the mean VAS score for "relieved" from 51.2 ± 19.4 at baseline to 65.8 ± 17.0 in week 8, indicating improvement of mood among the subjects. The VAS scores for other items ("feeling refreshed", "comfortable", "pleasurable", "light-hearted", and "relaxed") also increased in weeks 4 and 8.

In study 2, the VAS score for "pleasurable" was significantly higher in the singing group compared to the non-singing group. The VAS scores of the other items ("relieved", "feeling refreshed", "comfortable", "light-hearted", and "relaxed") were also higher at week 4 in the singing group compared with the non-singing group, but the differences were not significant.

Discussion

Good oral function is critical for various fundamental daily activities, both biological and social. Impairment of oral function, such as the ability to chew or swallow food, not only impacts the quality of life but can also lead to serious illness or require invasive medical interventions (such as insertion of a feeding tube). Deterioration of oral function can arise from organic or psychological causes. Psychological stress can exacerbate many diseases, while many disease processes can be better controlled by reducing stress [25,26]. Therefore, it is essential to maintain proper oral function and reduce psychological stress. It has been reported that music therapy is effective for pain [27] and for autism spectrum disorder [28]. In addition, it was reported that singing improves respiratory function in patients with chronic neurological conditions [29]. In this study, we investigated the efficacy of singing as a method of reducing psychological stress and improving oral function.

From middle age, there is a decline of the ability to swallow saliva and chew food. Our open-label study (study 1) showed that singing led to significant improvement of the RSST in weeks 4 and 8, while evaluation of chewing (by the bite force test assessing the strength of the masticatory muscles) revealed an increase of muscle strength in week 8. Thus, participants in study 1 showed improvement of oral function after 8 weeks of singing 4 times per week. In our cross-over study (study 2), the singing group showed significant improvement of the secretion of saliva (unstimulated saliva test) in week 4 compared with the non-singing group. Salivary glands are supported by the muscles of the oral cavity, and singing both strengthens these muscles and promotes local blood flow, which may explain how singing promotes saliva production. Reduction of saliva production, swallowing, and chewing are risk factors for aspiration pneumonia. Therefore, the present findings suggest that singing not only improves oral function, including saliva production and chewing, but may also reduce the risk of aspiration pneumonia.

The sympathetic and parasympathetic nervous systems are interrelated and exquisitely balanced components of the autonomic nervous system, which have a considerable impact on immune function (e.g., lymphocyte activity) [30]. Granulocytes increase when stress increases and sympathetic activity is predominant for an extended period. Conversely, when stress declines and parasympathetic activity becomes predominant, the number of lymphocytes (immunocompetent cells) increases. We observed a significant increase of lymphocytes after singing, which suggests that singing may influence the autonomic

Table 4. Swallowing and oral function in study 2.

		Non-singing group								Singing group								Analysis of variance
		Baseline			week 4			Change			Baseline			week 4			Change		
RSST	(times)	4.2	±	1.1	4.2	±	1.1	0	±	0.6	3.9	±	0.9	4.1	±	1.1	0.2	±	0.9	0.38
Bite force test	(kN)	0.056	±	0.029	0.061	±	0.02	0	±	0	0.1	±	0.035	0.063	±	0.029	0.004	±	0.03	0.824
Unstimulated saliva test	(mL)	4	±	2.8	3.7	±	2.1	-0.3	±	1.4	3.5	±	2.3	4.1	±	2.2	0.6	±	1.3	0.013
Saxon test	(g)	5	±	2	5.2	±	2.1	0.2	±	0.7	5	±	2.2	5.3	±	1.8	0.3	±	0.8	0.839
gum test	(mL)	17.8	±	7.4	17.3	±	7.6	-0.5	±	1.5	18	±	7.6	18.9	±	7.6	1.3	±	3.7	0.115

Data are shown as the mean± SD (n=17).
RSST, repetitive saliva swallowing test; SD, standard deviation

Table 5. Immune function parameters in study 1

Item		Baseline			week 4			week 8		
Immunity score		14.2	±	1.5	15.4	±	1.7[**]	15.8	±	1.9[**]
Immunity grade		2.7	±	0.5	3.0	±	0.4[*]	3.0	±	0.6
T lymphocyte age		59.7	±	11.4	58.7	±	11.8	56.8	±	14.2
CD3+T cells	(%)	64.8	±	9.2	66.5	±	8.3	68.5	±	8.3[*]
CD20+B cells	(%)	15.1	±	4.8	16.6	±	4.9[*]	14.4	±	5.7
CD4+T cells	(%)	45.6	±	8.2	46.5	±	6.1	47.1	±	7.4
CD8+T cells	(%)	14.6	±	5.3	16.7	±	6.0	18.8	±	7.1
CD4+ naïve T cells	(%)	33.5	±	7.0	40.1	±	10.6[**]	37.9	±	10.9[*]
CD4+ memory T cells	(%)	66.5	±	7.0	59.9	±	10.6[**]	62.1	±	10.9[*]
CD8 T cell subset CD28+	(%)	64.2	±	18.5	65.2	±	16.5	66.5	±	20.8
CD56+/16- NK cells	(%)	6.4	±	3.1	5.7	±	4.2	6.3	±	6.2
CD56+/16+ NK cells	(%)	13.7	±	7.4	12.9	±	6.8	13.3	±	7.4
CD56-/16+ NK cells	(%)	2.0	±	1.8	1.2	±	0.7	0.6	±	0.3[**]
Neutrophils	/μL	3117.3	±	1357.4	2828.5	±	1435.2	2710.1	±	1057.2
Lymphocytes	/μL	1631.7	±	470.4	1576.5	±	415.1	1611.2	±	310.9
T cells	/μL	1070.9	±	401.5	1056.2	±	334.0	1106.4	±	263.4
B cells	/μL	247.9	±	261.2	445.6	±	98.6	229.9	±	88.5
CD4+ T cells	/μL	747.1	±	274.0	735.1	±	215.0	762.7	±	202.3
CD8+ T cells	/μL	238.3	±	100.2	257.5	±	89.4	293.8	±	106.6
CD4+ /CD8+ T cell ratio		3.8	±	2.7	3.6	±	3.2	3.4	±	3.4
Naïve T cells	/μL	253.4	±	115.7	301.0	±	142.6[**]	285.8	±	107.2
Memory T cells	/μL	493.7	±	175.4	434.1	±	123.7	476.8	±	172.3
Naïve /memory T cell ratio		0.5	±	0.2	0.7	±	0.3[**]	0.7	±	0.3[*]
NK cells	/μL	215.6	±	124.7	200.1	±	107.0	210.6	±	117.2
CD8+/ CD28+ cells	/μL	144.0	±	58.8	161.2	±	58.1	192.5	±	91.9[*]

Data are shown as the mean± SD (n=13).
*p<0.05, ** p<0.01 : Wilcoxon signed-rank test vs. Baseline.
SD, standard deviation

Table 6. Changes of hormones and oxidative parameters in study 1

Item		Baseline			week 4			week 8		
Blood cortisol	(μg/dL)	7.08	±	1.32	6.31	±	1.29	6.04	±	1.48[*]
Blood adrenaline	(ng/mL)	0.040	±	0.020	0.033	±	0.016	0.030	±	0.021[*]
Blood noradrenaline	(ng/mL)	0.514	±	0.282	0.505	±	0.247	0.418	±	0.142
Blood lipid peroxide	(nmol/mL)	3.12	±	0.57	2.74	±	0.47[**]	2.86	±	0.57
Urine 8-oxodG	(ng/mg creatinine)	8.802	±	9.316	8.437	±	6.488	6.651	±	4.319

Data are shown as the mean± SD (n=13).
*p<0.05, ** p<0.01: Wilcoxon signed-rank test vs. Baseline.
8-OHdG, 8-hydroxy-2'-deoxyguanosine; SD, standard deviation

Table 7. Changes of hormones and oxidative parameters in study 2

		Non-singing group								Singing group								Analysis of variance
		Baseline			week 4			Change			Baseline			week 4			Change		
Cort	(µg/dL)	8.25	±	2.67	7.43	±	3.45	-0.82	±	3.55	8.00	±	2.27	7.94	±	2.91	-0.06	±	2.67	0.481
AD	(ng/mL)	0.021	±	0.013	0.021	±	0.011	0.000	±	0.008	0.023	±	2.290	0.022	±	0.016	-0.001	±	0.009	0.903
NA	(ng/mL)	0.443	±	0.146	0.499	±	0.168	0.056	±	0.108	0.441	±	0.130	0.531	±	0.201	0.091	±	0.132	0.205
LP	(nmol/mL)	3.10	±	0.46	2.81	±	0.53	-0.29	±	0.42	3.12	±	0.49	2.86	±	0.44	-0.25	±	0.29	0.744
8-OH	(ng/mg creatinine)	6.931	±	3.532	8.109	±	5.758	1.178	±	3.755	7.705	±	3.592	6.977	±	4.454	-0.729	±	3.009	0.128

Data are shown as the mean± SD (n=17).
Cort, blood cortisol; AD, blood adrenaline; NA, blood noradrenaline; LP, blood lipid peroxide; 8-OH, urine 8-hydroxy-2'-deoxyguanosine;
SD, standard deviation.

Table 8. Results of the questionnaire in study 1 (VAS)

	Baseline			week 4			week 8		
Refreshed	53.1	±	20.3	55.1	±	21.1	61.9	±	16.0
Comfortable	53.2	±	16.5	54.3	±	19.5	61.0	±	14.6
Pleasurable	51.5	±	22.6	58.4	±	21.1	59.0	±	20.6
Light hearted	49.8	±	21.7	57.5	±	22.8	57.5	±	20.0
Relieved	51.2	±	19.4	53.4	±	18.4	65.8	±	17.0*
Relaxed	53.5	±	20.7	59.8	±	21.5	64.3	±	16.6

Data are shown as the mean± SD (n=13).
*p<0.05, ** p<0.01: Wilcoxon signed-rank test vs. Baseline.
VAS score 0: Not At all, VAS score 100: Extremely
SD, standard deviation; VAS, Visual Analog Scale
The subjects marked the position that fitted their current state on a 100 mm line

Table 9. Results of the questionnaire in study 2 (VAS)

	Non-singing group									Singing group									Analysis of variance
	Baseline			week 4			Change			Baseline			week 4			Change			
Refreshed	61.9	±	19.2	63.9	±	18.7	2.0	±	22.1	55.8	±	20.8	64.7	±	12.9	8.9	±	21.4	0.422
Comfortable	59.4	±	16.5	63.0	±	20.2	3.6	±	19.8	56.4	±	21.7	63.9	±	14.8	7.5	±	25.3	0.623
Pleasurable	66.0	±	18.1	56.7	±	18.8	-9.2	±	17.8	56.6	±	19.5	65.6	±	14.6	9.0	±	21.0	0.035
Light hearted	53.1	±	22.0	64.0	±	20.9	10.9	±	28.9	55.0	±	24.2	62.9	±	19.4	7.9	±	30.8	0.706
Relieved	62.1	±	20.6	61.0	±	18.0	-1.1	±	22.0	56.5	±	19.6	66.2	±	19.9	9.8	±	21.3	0.239
Relaxed	61.4	±	20.7	61.4	±	19.7	0.0	±	22.1	56.5	±	23.0	67.0	±	18.2	10.4	±	24.0	0.293

Data are shown as the mean± SD (n=13).
VAS score 0: Not At all, VAS score 100: Extremely

balance. In week 8 of study 1, there was a significant decrease of adrenaline and cortisol levels (which are elevated by stress), and the questionnaire results suggested that stress was reduced among the participants. In study 2, the questionnaire results showed improvement in the singing group compared with the non-singing group, although blood levels of adrenaline and cortisol did not differ between the two groups. These results combined with immunological data (study 1) suggest that singing reduces stress and improves immune function.

Oxidative stress is influenced by mental health and environmental factors, and this type of stress may play a role in the onset of various diseases, including type II diabetes and cancer [31]. Cell membranes are composed of unsaturated fatty acids, and lipid peroxides can easily damage these molecules, potentially leading to disease [32]. Moreover, In addition, 8-OHdG has various pathologic effects and its urine level is measured as an indicator of oxidative stress [33]. In study 1, we observed a significant decrease of lipid peroxide in week 4 and urine levels of 8-OHdG also decreased over the 8-week study period. In study 2, the urine 8-OHdG level decreased in the singing group while increasing in the non-singing group, although there was no significant difference between the two groups. These findings suggest that singing may have the potential to prevent various diseases by reducing oxidative stress. In this context, it was previously demonstrated that production of reactive oxygen species in rats subjected to immobilization stress was decreased by eating behavior, including chewing [34, 35].

Because this small-scale study showed that daily singing improves psychological stress and mental health, further investigation is warranted to evaluate the effect of singing in a larger study.

Conclusions

In our 2 studies, singing on a regular basis for 1-2 months increased secretion of saliva, as well as leading to improvement of chewing and swallowing. Singing therapy was also associated with reduction of psychological stress, and may potentially prevent/delay various diseases by reducing oxidative stress and improving immune function.

Author Contributions

KS conceived the study, participated in the study design, performed data collection, and drafted the manuscript. KR, YT, RN, and SF performed statistical analysis.

SE participated in the study design and performed data collection. KT participated in the study design. IS reviewed the study. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests and these data have not been published or used before in any manner in a journal article.

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