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Effect of Vitamin B_{12} Injection on the Vocal Performance of Professional Singers

A Randomized, Double-blind, Placebo-Controlled, Crossover Trial

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IMPORTANCE One-third of singers and vocal professionals report experiencing a benefit from empirical vitamin B_{12} injections for improvement of mild singing-related symptoms (eg, reduced stamina, vocal fatigue, and effort). However, there is no objective evidence to support or refute these claims.

OBJECTIVE To assess the presence and magnitude of the effect of empirical vitamin B_{12} injection on the vocal performance of singers.

DESIGN, SETTING, AND PARTICIPANTS A randomized, double-blind, placebo-controlled, crossover trial was conducted from November 7, 2017, to November 30, 2018, at an academic voice center among 20 active adult singers without dysphonia but with mild vocal symptoms. Individuals with known or suspected vitamin B_{12} deficiency or active or recent vitamin B_{12} treatment were excluded. Analysis was on a per-protocol basis.

INTERVENTIONS Participants were randomized to receive an intramuscular (deltoid) injection of either vitamin B_{12} (1000 µg of cyanocobalmin) or placebo (0.9% sodium chloride). After a washout period of at least 4 weeks, participants were crossed over to receive the opposite injection. Both the investigators and participants were blinded to the order of injections.

MAIN OUTCOMES AND MEASURES The participants completed the Singing Voice Handicap Index–10 (SVHI-10), the Voice Fatigue Index (VFI), and the Evaluation of the Ability to Sing Easily (EASE) before each injection and at intervals of 1 hour, 3 hours, 24 hours, 72 hours, and 1 week after the injection. The primary time point assessment was 72 hours after injection, and the SVHI-10 score was the primary outcome measure.

RESULTS Twenty singers (10 men; median age, 22 years [range, 19-42 years]) were enrolled. The improvements after either placebo or vitamin B_{12} injections were comparable to each other. At 72 hours after the vitamin B_{12} injection, the median difference in the SVHI-10 score was 1 (95% CI, −1 to 2) compared with 3 (95% CI, 0-4) after placebo. The median difference between differences at 72 hours between placebo and vitamin B_{12} injections were 1.5 (95% CI, −2 to 5) for the SVHI-10, 1 (95% CI, −9 to 9) for the VFI, and −1 (95% CI, −3 to 2) for the EASE. The improvements after both injections failed to reach the estimated minimal clinically important difference. Of the 20 participants, 4 (20%) reached the estimated minimal clinically important difference in their SVHI-10 score after 72 hours for both vitamin B_{12} and placebo injections.

CONCLUSIONS AND RELEVANCE This randomized, double-blind, placebo-controlled, crossover trial found that after empirical vitamin B_{12} injection to improve mild voice-related symptoms, the improvement in self-reported voice measures in singers shows no meaningful difference compared with placebo.

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Supplemental content

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Vitamin B_{12}, also referred to as cobalamin, is an important nutrient required to carry out essential functions, such as erythropoiesis and metabolic conversion of proteins and fat, and to support the normal function and development of nerve cells.[1] Vitamin B_{12} deficiency is common, and about 6% of individuals 60 years of age or older are vitamin B_{12} deficient.[2] Manifestations of vitamin B_{12} deficiency are extensive and associated with fetal and childhood development and have ramifications for elderly individuals.[1,3] Commonly, vitamin B_{12} deficiency is associated with megaloblastic anemia and neurologic conditions.[4,5] Other manifestations include mood disorders such as depression,[6-8] osteoporosis, gastrointestinal symptoms (eg, glossitis, decreased appetite, and constipation), and skin lesions (eg, hyperpigmentation, vitiligo, and hair changes).[9,10]

The scientific literature connecting vitamin B_{12} with laryngeal disorders or function is limited, to our knowledge. Vitamin B_{12} deficiency was suggested to be associated with chronic cough and laryngeal hyperresponsiveness.[11] There have been reported cases of vocal fold paralysis as a neurologic manifestation of vitamin B_{12} deficiency,[12,13] and there are some sparse reports that metabolic alterations in homocysteine, folate, and vitamin B_{12} levels are associated with laryngeal carcinogenesis and leukoplakia.[14,15]

Singers and voice professionals often seek complementary and alternative treatments to improve or enhance their vocal function. A recent survey study[16] revealed a common belief among singers that there are some vocal benefits from empirical vitamin B_{12} treatment (treatment given without the knowledge of a cause or an underlying condition). Of the surveyed singers, 31% stated that they believe vitamin B_{12} therapy improves vocal performance, and 33% of the surveyed fellowship-trained laryngologists reported that they have been asked by a singer to prescribe vitamin B_{12} for voice benefits. The survey results also revealed a discrepancy between the singers' and the laryngologists' perspectives. Although 31% of the surveyed singers believed that vitamin B_{12} therapy improves vocal performance, none of the laryngologists did. This discrepancy between singers and physicians is most probably due to the lack of evidence on the effect of vitamin B_{12} on vocal performance and the possibility that reported voice benefits may be attributed to the placebo effect alone. Reliable evidence is needed to either support or reject the use of empirical vitamin B_{12} injections to improve vocal performance. The use of any medication, including vitamin B_{12}, may involve risks, adverse effects, and toxic effects that are unacceptable when the medication is not indicated.

In this study, we sought to assess the presence and magnitude of the effect of empirical vitamin B_{12} injection on the self-reported vocal performance of singers by means of a prospective double-blinded, randomized, placebo-controlled, crossover trial. The effects were estimated using patient-reported outcome (PRO) measures, with the Singing Voice Handicap Index-10 (SVHI-10)[17] as the primary outcome measure. The study was designed to mimic the clinical scenario of a singer requesting an empirical vitamin B_{12} injection to improve mild voice-related symptoms without a known vitamin B_{12} deficiency and without the availability of laryngoscopy.

Key Points

Question Do singers and voice professionals experience voice benefits from empirical vitamin B_{12} injection for improvement of mild singing-related symptoms, such as reduced stamina, vocal fatigue, and effort?

Findings In this randomized, double-blind, placebo-controlled, crossover trial, the 20 singers who participated in the study failed to demonstrate improvements in self-reported vocal performance measures after vitamin B_{12} injection that were different from those observed after placebo injection.

Meaning After empirical vitamin B_{12} injection, the improvement in self-reported voice measures in singers is no different from that with a placebo injection.

Methods

This is a randomized, double-blind, placebo-controlled, crossover study, conducted from November 7, 2017, to November 30, 2018, at the University of Southern California (trial protocol in Supplement 1). After approval by the University of Southern California (USC) institutional review board, individuals were recruited to participate through advertisements displayed at the USC Voice Center clinics (Keck Medical Center Otolaryngology Clinic, Keck Medicine Downtown Los Angeles Clinic, and USC-Glendale Otolaryngology Clinic), at the USC Thornton School of Music facilities, and on social media (Facebook: USC Voice Center, USC School of Music, Voice Forum, Singer Friends LA, and The Boston Conservatory Alumni). A copy of a recruitment ad can be found in eFigure 1 in Supplement 2. Participants provided written informed consent. This study followed the Consolidated Standards of Reporting Trials (CONSORT) reporting guideline.

Inclusion and Exclusion Criteria

To qualify for the study, individuals had to fulfill all of the following inclusion criteria: (1) active vocational singers, (2) aged 18 to 65 years, and (3) willing to comply with study requirements (eg, adherence to scheduled visits, receiving both injections, and completing the questionnaires on schedule). The exclusion criteria included (1) known vitamin B_{12} deficiency, (2) active or recent vitamin B_{12} treatment in the last 4 weeks, (3) bleeding disorders, (4) history of laryngeal pathologic conditions, (5) current dysphonia (because the study was designed to assess enhancement of vocal performance rather than treatment of dysphonia), and (6) plans to begin any new treatment, medication, or supplements. Although recruited participants did not have dysphonia, they had mild voice-related symptoms such as reduced stamina, vocal fatigue while performing, or increased singing effort. In addition, individuals who scored in the top tenth percentile of the SVHI-10, the Evaluation of the Ability to Sing Easily (EASE),[18] and the Voice Fatigue Index (VFI)[19] were excluded owing to the inability to

demonstrate improvement according to the study's outcome measures. Individuals who were found eligible were enrolled in the study between November 7, 2017, and November 30, 2018.

Study Protocol

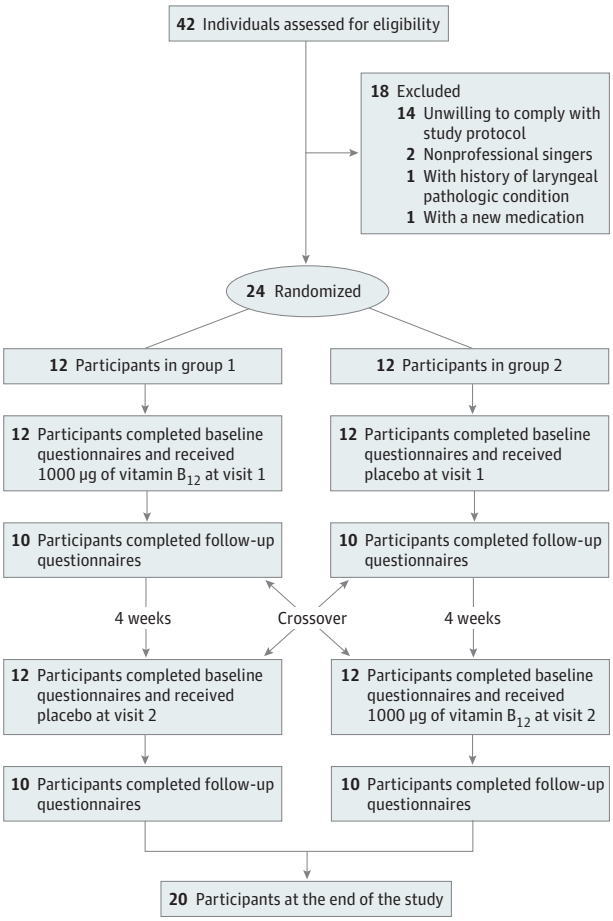
Enrolled participants were randomized in a 1:1 ratio, using a sealed envelope system, to determine the order of injections based on their designated treatment group (group 1 or group 2; **Figure**). The envelopes were opened by a research assistant, one at a time for each study participant enrolled. Only the research assistant and the nursing staff member, who prepared and administered the injections, were aware of the group of randomization. Both the clinician or caregiver and the participants were blinded to the randomization. Participants were required to visit the clinic on 2 separate occasions, at least 4 weeks apart, and receive 1 injection during each visit. On first visit arrival, the inclusion and exclusion criteria were rereviewed, participants' demographic information was collected, and informed consent was signed in the presence of the principal investigator.

Prior to receiving each injection, participants completed the following PRO measures to gauge vocal state at baseline: the SVHI-10 questionnaire[17] (the primary outcome measure), the VFI questionnaire,[19] and the EASE questionnaire.[18] Next, the participants received an intramuscular injection in the deltoid muscle. The injectable was determined by the participants' treatment group (group 1 or group 2; Figure), consisting of either 1000 µg of cyanocobalamin (vitamin B_{12}) or 0.9% sodium chloride solution (as placebo). The injected material was concealed (for blinding) owing to the color difference between cyanocobalamin and sodium chloride solution.

After each injection, the participants were asked to repeat the PRO measures for 5 more times, at intervals of 1 hour, 3 hours, 24 hours, 72 hours, and 1 week after the injection. The preinjection and 1-hour postinjection PRO measures were completed in the office during the injection visit. For all other PRO measure completion time points, participants were reminded via text messages on their personal cellular telephone. The timeline for the PRO measures and for the interval between the 2 injections was based on the pharmacokinetics of vitamin B_{12}.[20] The serum level of vitamin B_{12} peaks about 1 hour after an intramuscular injection. More than 50% is extracted within 48 hours, most of it in the first 8 hours. The time point of 72 hours after injection was chosen for the primary postinjection time point assessment. The first 3 postinjection time points, within the first 24 hours, were used to assess the immediate effect of the injection. The 1-week postinjection time point was added to evaluate the durability of the effect. At a dosage of 1000 µg, up to 15% of the vitamin B_{12} is stored (primarily in the liver). For patients with vitamin B_{12} deficiency treated with vitamin B_{12} injections, a maintenance dose is given once a month to maintain serum levels. Thus, a 4-week minimum washout period between injections was selected.

The change in SVHI-10 score before the injection and 72 hours after the injection was chosen as the primary outcome measure. The voice-related PRO measures were selected because they were all designed to assess the vocal performance of singers and because each PRO measure represents different aspects of the singing voice. However, none of the singing-specific PRO measures has a known minimal clinically important difference (MCID). The SVHI-10 was chosen as the primary outcome measure because it is widely used and because it has a design and a scale similar to a well-established PRO measure, the Voice Handicap Index 10.[21,22] Although the Voice Handicap Index 10 does not assess the singing voice, its MCID was determined by previous studies to be between 4 and 6. Considering the Voice Handicap Index 10 as a reference, our study design predetermined an MCID of 5 for the SVHI-10.

Figure. Flow Diagram Describing the Progress of Patients Throughout the Clinical Trial



Statistical Analysis

Analysis was performed on a per-protocol basis. Because this is the first study, to our knowledge, to investigate the effects of vitamin B_{12} on vocal performance, the sample size was determined based on estimation alone, assuming the trial will result in a medium effect size (0.5) and for a 2-tailed α of 0.05 with a power of 0.9 (for detecting a MCID of 5 for the SVHI-10), while comparing 2 normally distributed paired groups (*t* test). The power calculation resulted in a sample size enrollment of at least 18 individuals for a crossover study. The power

Table 1. Description of the General Characteristics and Demographic Charateristics of the Study Population

Characteristic	Participants, No. (%)		
	Group 1 (n = 10)	Group 2 (n = 10)	Total (N = 20)
Age, median (IQR), y	21 (28.5-20)	21.5 (22-20)	22 (25-20)
Male to female ratio	5:5	5:5	10:10
Smokers	1 (10)	0	1 (5)
Primary singing genre			
Classical	6 (60)	7 (70)	13 (65)
Musical theater	2 (20)	2 (20)	4 (20)
Pop	2 (20)	1 (10)	3 (15)
Singing experience, median (IQR), y	15 (20.5-7.5)	13 (18.5-8.75)	13 (20-8)
Formal singing training	10 (100)	9 (90)	19 (95)
Mean amount of singing, median (IQR), h/wk	10 (10.5-8)	13 (18.75-8.75)	10 (14-8)
Voice type			
Soprano	2 (20)	3 (30)	5 (25)
Mezzo-soprano	3 (30)	2 (20)	5 (25)
Bass	3 (30)	0	3 (15)
Baritone	1 (10)	1 (10)	2 (10)
Tenor	1 (10)	3 (30)	4 (20)
Countertenor	0	1 (10)	1 (5)
Diet			
Omnivore	8 (80)	6 (60)	14 (70)
Semivegetarian or flexitarian	1 (10)	2 (20)	3 (15)
Vegan	1 (10)	1 (10)	2 (10)
Pescatarian	0	1 (10)	1 (5)
Taking a multivitamin	4 (40)	2 (20)	6 (30)

Abbreviation: IQR, interquartile range.

calculation was performed using G*Power, version 3.1.9.2 (Universität Kiel).

The collected data and the scores of each questionnaire were imported into a Microsoft Excel 2010, version 14 spreadsheet (Microsoft Corp). All statistical analyses were performed using SPSS Statistics software, version 25.0 (IBM Corp). The normal distribution assumption was performed by exploring each parameter for mean, median, mode, skewness, and kurtosis. Because the scores of each of the PRO measures failed to demonstrate a normal distribution, they were described by median values and ranges, and nonparametric tests for repeated measures were applied to test the difference between repeated ranking measurements.

For each PRO measure under each study group, we have calculated the change in the PRO measure from baseline and after 72 hours and the difference in the difference between groups. Because the distribution of the calculated difference within and between each study group failed to demonstrate a normal distribution, the median value and 95% CI were used for description, and the Wilcoxon signed-rank test (nonparametric test equivalent to dependent *t* test) was calculated. Ranking analysis uses pooled ranking of all observed differences between 2 dependent and repeated measurements. Effect size was extracted using the distributed *z* value to report the magnitude and direction of the differences for the various outcome measures. Confidence intervals were calculated for absolute values and for the differences of the measures.

Results

Of 42 candidates, 24 met the study inclusion and exclusion criteria. On their first visit, all 24 enrolled participants scored less than the top tenth percentile of the baseline questionnaires (SVHI-10, EASE, and VFI), and therefore all continued to the next steps of the study. Participants were evenly randomized into 2 groups. After randomization, 4 participants (1 man and 1 woman from each group) failed to complete the first visit follow-up questionnaires and failed to return for their second clinical visit, and therefore they were excluded from the study. Altogether, 20 participants—10 in each group—completed the entire study protocol (Figure). Of the 20 study participants, 10 (50%)—5 in each group—were male. The median age of the study population was 22 years (range, 19-42 years), with comparable distribution in each group. The primary singing genre was classical for 13 participants, musical theater for 4, and pop for 3. A further description of the study population is presented in **Table 1**. No adverse events or adverse effects related to the injections were reported by the study participants.

As demonstrated in **Table 2** and eFigure 2 in Supplement 2, all PRO measures failed to show the superiority of vitamin B_{12} vs placebo. The improvements after either placebo or vitamin B_{12} injections were comparable to each other. At 72 hours after the vitamin B_{12} injection, the median difference

Table 2. Comparison of Differences in the Median Scores and Ranks Before and After Placebo and Vitamin B_{12} Injections

	Placebo injection				Vitamin B_{12} injection				Comparison	
	Median (range)		Difference B_{12}, median (95% CI)[a]	Effect size	Median (range)		Difference placebo, median (95% CI)[a]	Effect size	Difference between differences, median (95% CI)[b]	Effect size
Questionnaire	Before	72 h After			Before	72 h After				
SVHI-10	10.5 (0 to 29)	8.5 (0 to 16)	3 (0 to 4)	−0.52	10 (0 to 21)	8 (0 to 19)	1 (−1 to 2)	−0.21	1.5 (−2 to 5)	−0.31
VFI	24 (4 to 43)	22 (0 to 45)	1.5 (−1 to 6)	−0.34	25 (9 to 45)	21 (5 to 46)	0 (−1 to 7)	−0.21	1 (−9 to 9)	−0.10
EASE	14 (8 to 31)	12 (9 to 22)	1 (−1 to 5)	−0.51	19 (10 to 34)	14 (8 to 29)	2 (−1 to 5)	−0.41	−1 (−3 to 2)	−0.01

Abbreviations: EASE, Evaluation of the Ability to Sing Easily; SVHI-10, Singing Voice Handicap Index-10; VFI, Voice Fatigue Index.

[a] From before the injection to 72 h after the injection.

[b] Vitamin B_{12} difference − placebo difference.

or improvement in the SVHI-10 score was 1 (95% CI, -1 to 2) compared with 3 (95% CI, 0-4) after placebo. The median difference between differences at 72 hours after vitamin B_{12} injection compared with placebo were 1.5 (95% CI, -2 to 5) for the SVHI-10, 1 (95% CI, -9 to 9) for the VFI, and -1 (95% CI, -3 to 2), for the EASE. Moreover, these improvements after both injections failed to reach clinical significance. As demonstrated in Table 2, the 95% CI of the median score differences for the SVHI-10 after either vitamin B_{12} or placebo did not reach the predetermined MCID (5 points). Of the 20 participants, 4 (20%) reached an improvement of at least 5 points in their SVHI-10 score after 72 hours for both the vitamin B_{12} and placebo injections. The results of the 72-hour postinjection time point are detailed in Table 2, and the results of all other postinjection time points are given in eFigure 2 in Supplement 2.

Discussion

With limited data regarding the effect of vitamin B_{12} treatment on vocal performance, clinicians cannot effectively counsel vocal performers requesting empirical vitamin B_{12} injections to enhance vocal capabilities. As was recently published, this scenario is not uncommon because one-third of 64 surveyed laryngologists reported that they have been asked by a singer to prescribe vitamin B_{12} for voice benefits.[16] In our randomized, double-blind, placebo-controlled, crossover trial, 20 singers without vitamin B_{12} deficiency were injected with vitamin B_{12} and placebo on 2 separate occasions. The participating singers demonstrated improvements in scores on different vocal function-related questionnaires after either vitamin B_{12} or placebo injection. However, the improvements that followed vitamin B_{12} injection failed to demonstrate superiority over the improvements that followed placebo injection.

Placebo and Placebo Effect

To verify the efficacy of treatment, a therapy should be proven to be significantly better than placebo.[23] Although vitamin B_{12} demonstrated improvements compared with preinjection scores, these improvements were not different from those observed after placebo injection. The findings of our study suggest that there are no voice-related benefits after vitamin B_{12} injection and that vitamin B_{12}-related effects are comparable to those seen with placebo. Placebo is defined as a sham medication or procedure designed to be void of any known therapeutic value.[24] Placebo effect is the positive response some may experience after receiving placebo. Placebo effects are believed to be related to intrinsic factors (eg, personal expectations) or extrinsic factors (eg, interaction with the clinician). It is agreed that placebo can affect disease symptoms that depend on patients' perception. For example, a recent meta-analysis indicated that placebo may be associated with improved perceived insomnia symptoms, with no effect on objective measures, such as sleep-onset latency.[25] A Cochrane study investigating placebo interventions for 60 clinical conditions did not find any important clinical effects caused by placebo but stated that placebo may be associated with PRO measures related to pain and nausea.[26] The voice-related outcome measures in our study all rely on self-perception and are therefore subject to placebo effects. The outcome measures in our clinical trial trended in a comparable direction and magnitude after both placebo and vitamin B_{12} injections, as all indicated minor improvements.

The placebo effect on professional singers' performance is well demonstrated in our study (after both injections). Professional singers can be appreciated as vocal athletes, with extremely high vocal demands, pushing their vocal capabilities to their upper limit. It is therefore possible to relate this placebo effect to the placebo effect in sport athletes, who have been previously studied and are better understood. In a survey of 79 elite athletes, 47% reported that they have experienced performance placebo effects in the past, and 82% believe that placebo can positively affect their performances.[27] A meta-analysis reviewing 196 sport athletes concluded that placebo has a small to moderate effect on sports performance.[28] A clinical trial of 43 competitive endurance cyclists found that the effect of carbohydrate drinks was reduced when the athletes were told it was placebo, while the effect of placebo was improved when the athletes were told it was a carbohydrate drink.[29] Similarly, the velocity of Paralympic weightlifting was improved after athletes took a placebo capsule while informed that it contained caffeine.[30]

Importance

Based on the findings of our clinical trial, it can be recommended that vitamin B_{12} should not be given empirically to improve the vocal performance of singers and voice profes-

sionals. Moreover, it is possible to extend this recommendation and state that vitamin B_{12} should not be given empirically to treat dysphonia.[31] Our study did not directly assess the participants' vitamin B_{12} serum level; nevertheless, singers with known or suspected vitamin B_{12} deficiency were excluded from the study. Consequently, the study did not investigate whether singers with vitamin B_{12} deficiency can experience true vocal benefits from vitamin B_{12} treatment. We advise that vitamin B_{12} deficiency should be treated in singers as a medical condition that requires therapy. Whether these singers with vitamin B_{12} deficiency experience vocal benefits from vitamin B_{12} treatment and a correction of the deficiency should be investigated in further studies. Ideally, these further studies should measure vitamin B_{12} serum levels and clearly distinguish between singers with vitamin B_{12} deficiency and those with normal vitamin B_{12} levels, which was not performed in the present study.

The importance of avoiding the use of ineffective treatment is mostly related to possible adverse events, such as adverse effects, drug interactions, overdose, and toxic effects. Vitamin B_{12} is considered a relatively safe medication, even when a very high dosage is used. Nevertheless, anaphylactic shock has been reported,[20,32] and specific conditions warrant caution (eg, patients with Leber disease may develop severe optic atrophy). We therefore argue that, although vitamin B_{12} is considered safe and none of our participants experienced any drug-related adverse events, the unnecessary use of vitamin B_{12} should be avoided.

Limitations

This study has some limitations. Owing to the importance of the singers' perspective, the study's primary and secondary outcome measures are patient reported. However, a correlation with objective voice measures is still required and should be further investigated. Moreover, the study protocol required completing each questionnaire repeatedly within 1 week. These measures were not designed for repeated administrations within a few hours per day. A possible learning effect (associated with remembering the previous answers) or recall issues may have resulted in biased outcomes and should be taken into consideration. In particular, the questionnaires were completed after 3 injections within 24 hours.

The study was designed to look for the positive effects of empirical vitamin B_{12} injection on vocal performance, not for negative effects. Hence, according to the study protocol, individuals who, prior to injections, scored in the top tenth percentile of the SVHI-10, VFI, and EASE were excluded. Nevertheless, eventually 95% of the participants scored between the top 17th and 30th percentiles, which can potentially allow for detection of both positive and negative effects. Properly designed randomized clinical trials are uncommon in the fields of laryngology and care for the professional voice. The findings of our study failed to demonstrate any clinically significant benefits caused by vitamin B_{12} compared with placebo. Although this is a properly designed randomized clinical trial, it can be reasoned that the study was underpowered to detect possible minor changes. Nevertheless, we argue that a larger-scale trial would reveal only minor differences, which would have no clinical significance. Hence, this should not affect the legitimacy of our recommendation that vitamin B_{12} should not be given empirically to improve the vocal performance of singers and voice professionals.

Conclusions

After empirical vitamin B_{12} injection for mild voice-related symptoms, changes in self-evaluation of vocal performance are no greater than those seen with placebo and fail to reach clinical significance. It is therefore recommended that vitamin B_{12} should not be given empirically to improve the vocal performance of singers and voice professionals.

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