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Supporting the Health of College Solo Singers: The Relationship of Positive Emotions and Stress to Changes in Salivary IgA and Cortisol during Singing

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## Supporting the Health of College Solo Singers

Although singers appear to experience health benefits from singing, the performance of their art also makes physical demands that may leave them prone to health problems. Two lines of research are reviewed: one supports the health benefits of singing, and the second claims that intensive singing may entail risk factors for air-borne infections and other health risks, including increased potential for reflux (morning hoarseness), emotional stress (insomnia), and upper respiratory tract infections (URTI). Health Benefits and Risk Factors in Singing

A variety of studies have suggested that singing results in significant health benefits for singers. For example, Kenny and Faunce<sup>1</sup> found a mild improvement in patients' coping mechanisms with pain after small-group singing. A study of amateur and professional singers in a single singing lesson showed that professionals had more cardio-physiological fitness and arousal, but amateurs reported more joy and elatedness.<sup>2</sup> In a questionnaire study, 58% of the members of a college choral society reported that they had benefited in some physical way, citing increased control over breathing, improved mood, and stress reduction.<sup>3</sup> Beck et al.<sup>4</sup> collected open-ended questionnaire responses on the benefits of music; many singers reported that singing was "healing," "stress-reducing," and "refreshing." An analysis of 50 years of articles in an important popular music magazine, *The Etude*, contained consistent and adamant support regarding the health benefits of singing.<sup>5</sup> In recent studies, teams of medical and social scientists have shown that adult choir singing is associated with transient increases in salivary Immunoglobulin A, (S-IgA).<sup>4,6</sup> IgA is the body's primary defense against pathogens attempting to penetrate or attach to the epithelial surfaces, such as the respiratory or gastrointestinal tract.<sup>7,8</sup> The implications of these studies and those showing upward

regulation of S-IgA after singing is that vocal performance may have a positive influence on health.

However, the demands of professional singing training may increase health risks. Cisler<sup>7</sup> argued that "the increased air flow experienced by singers and wind players could be a factor in the increase of S-IgA levels detected after playing or singing," and that "increased airflow could result in increased exposure to foreign antigens, and therefore a higher S-IgA count in response" (p. 6). In a study of the effects of intense exercise, which increases airflow, MacKinnon and Hooper<sup>9</sup> found that after an initial decrease on Day 1, S-IgA increased on Days 2 and 3. MacKinnon and Hooper also cited clinical and epidemiological studies suggesting that athletes had higher risks for infections at the mucosal level. In a survey of vocal health in young singers, Tepe et al.<sup>10</sup> found that half of their sample (n = 129) reported experiences of vocal difficulties, particularly reflux (morning hoarseness) and emotional stress (insomnia). However, levels of upper respiratory tract infection (URTI) were not measured. In a year-long longitudinal study of football players, Fahlman and Engels<sup>11</sup> found a strong relationship between overall decreases of S-IgA and the secretion rate of S-IgA with the prediction of incidence of URTI. Cisler<sup>7</sup> called for comparable studies of singers.

Thus, very preliminary research studies and informed speculation seem to indicate that singers experience both health benefits and problems from their artistic activity.

While singing appears to provide better control of breathing, stress reduction, and is often cited as providing both healing and spiritual benefits that promote health, the very demands of everyday singing training may also leave singers open to risk factors. To assess both benefits and risks in young singers, the present study was undertaken to measure variables that both contributed to and diminished immune competence in

response to singing under different conditions of rehearsal and performance. Because previous research has demonstrated that S-IgA is an important marker for immune response, the present study will measure levels of this protein before and after singing in repeated measures from a sample of young singers.

Mediation of Immunocompetence by Psychological States

While researchers have shown that adult choir singing is associated with transient increases in S-IgA, <sup>4,6</sup> the upward changes were mediated by psychological variables. In a carefully controlled study, Kreutz et al.<sup>6</sup> demonstrated that active singing, and not passive listening, led to significant upward changes in IgA and positive mood. The principal conclusion of Beck et al.,<sup>4</sup> based on a regression analysis of their choral singers' responses, was that positive emotions, such as relaxation, feeling high, being engaged, having elevated mood, and satisfaction with performance, were predictive of significant upward changes in S-IgA. Other research on music has confirmed the influence of positive emotions and stress on the immune system, using S-IgA as a marker.<sup>3, 12, 13, 14</sup>

Singing in performance is likely to be stressful. Hucklebridge et al. <sup>15</sup> showed that stress reduced the upward regulation of S-IgA. Questionnaire data from Beck et al. <sup>4</sup> indicated that "31.8% agreed that singing was stressful or were non-committal. With respect to the performance, 43.6% of singers reported feeling anxious before singing" (pp. 102-103). They concluded that "immune system functioning is likely to be stimulated both as a response to anxiety and as a function of the pleasure of the singing experience" (p. 104). Hucklebridge et al. <sup>15</sup> also found that performance tasks representing acute challenges are in fact associated with transient increases in S-IgA. By contrast, in their earlier study, Hucklebridge et al. <sup>16</sup> reported that the level of S-IgA is down regulated during periods of chronic stress.

However, research on singing has not demonstrated consistent effects on levels of cortisol and has not always shown a relationship between IgA and cortisol in performance tasks. Hucklebridge et al. found that a rise in cortisol was significantly correlated with decreases in IgA in a 30-minute period for subjects after awakening, which is considered a physiologically stressful event. By contrast, in a mood manipulation study that used music to induce positive mood and mental recall to induce negative mood, Hucklebridge et al. later found no changes in cortisol related to either condition, despite finding significant rises in S-IgA. Thus, cortisol has been less reliable as a marker of performance-related immune functioning.

A Preliminary Model of the Relation of Soloists' Positive Psychological States and Performance Stress to Changes in Immunological Enhancement during Singing

The present study will consider whether both positive emotions and values on the one hand, and short-term and long-term performance stress on the other, will influence S-IgA production in solo singers' rehearsals and performance. This approach will also address the question of whether individual solo singers demonstrate the same effects of singing on their immunocompetence as those previously observed for choir singers. The effects of positive emotions in soloists are predicted to operate in the same manner as in choirs. However, the questions concerning the influence of stress on solo singing are more interesting, because choir singing is probably not accompanied by performance anxiety to the extent that it appears in individual performance. We also expected that individual singers would have more performance anxiety during performances in front of judges and an audience than in solitary rehearsals. Young performers who regard singing in career terms have necessarily set the bar higher and thus may have more anxiety than singers at a comparably early point in their singing experience who have a relatively

casual approach to the art. A professional singing career involves sustained and demanding activity, especially in the early years, in contrast to "amateur" performances that entail lower stakes and, consequently, lower stress.

From survey instruments used in previous research (Beck et al., 1999), we selected <u>positive emotional and values variables</u> that have previously been seen to relate to increased levels of S-IgA during rehearsal and performance singing. Given cortisol's uncertain relationship with performance demands, these variables might also be associated with decreased levels of cortisol:

- > relaxation
- > feeling high
- > mood rises before singing
- > well being
- > satisfaction with performance

Based on the survey instrument used in previous research<sup>4</sup> and the findings of Hucklebridge et al.<sup>15, 16</sup> about the relation of performance demands to chronic stress, we selected <u>stress variables</u> that might be expected to decrease levels of S-IgA and increase cortisol in response to performance demands:

- > usually stressed when singing
- > singing regarded as professional artistic activity

These psychological variables were used to form and test hypotheses concerning the impact of singing on S-IgA and cortisol.

#### Methods

# **Participants**

The singers (N = 10) were all music majors in a conservatory in a small liberal arts college. The students ranged in age from 18-25 years with a mean of 20.8 years. They had been singing an average of 10.1 years. None of the singers smoked nor consumed more than 10 alcoholic drinks per week.

Each singer collected her or his own saliva samples over a 10-week period. The number of pre- and post-singing samples successfully collected from each singer ranged from 4 to 11. Samples were delivered within 2 hours to a local refrigerator and subsequently transported to a freezer for storage and subsequent assay in a chemistry laboratory.

Saliva collection and Laboratory Test

Singers collected saliva samples immediately before and after each rehearsal and performance session. A sampling device, "Salivette" (Sarstedt, Inc., Rommelsdorf, Germany) was used for collecting saliva. The Salivette consists of a small cotton swab that fits inside a standard centrifugation tube. Subjects chewed gently on the swab for approximately 30-60 seconds. The Salivettes have been used in other saliva research and have been described as the most convenient device for self-administered saliva collection.<sup>17</sup>

The saliva samples were assayed using an ELISA procedure. Sandwich ELISA for S-IgA was performed with antibodies from Research Diagnostics (Concord, MA). Phosphate buffered saline – with 0.1% Tween 20 (PBST) was used for all washes. Cortisol ELISA was performed using a kit from Caymen Chemicals (Ann Arbor, MI). Absorbances were read at 450 nm. S-IgA and cortisol concentrations were calculated by interpolation from a standard curve, and all determinations were done in triplicate. Data

were summarized as the mean  $\pm$  SD of triplicate determinations when a representative is shown and mean  $\pm$  SEM for summaries of experiments on multiple participant samples. Statistical significance of differences was determined by the students' t test.

To standardize the results of the ELISA, a quantitative analysis of the protein present in each person's saliva was necessary. Every person's saliva is unique to them, and has unique protein levels. By standardizing the results of the ELISA, we were able to make comparisons among all saliva samples.

The Bradford assay kit (Biorad, Hercules, CA) to quantify the amount of protein present was used following manufacturer's instructions. Absorbances were read at 590 nm. Data were summarized as the mean  $\pm$  SD of triplicate determinations when a representative is shown and mean  $\pm$  SEM for summaries of experiments on multiple participant samples. Measurements were taken by a spectrophotometer, which reads the amount of light absorbed by the solution.

Rating Scales of Psychological Variables Associated with Singing

Questionnaire. A "Singers Emotional Experiences Scale" (SEES) was adapted from Beck et al.<sup>4</sup> The SEES consisted of 39 items, including 16 pairs of Likert-scale questions, posed affirmatively ("singing is important to me") and negatively ("singing is not important to me"), asking singers to rate emotions and values *typically* experienced during singing. Thus, the questions referred to long-term emotions and values that were personally associated by singers with their singing in general. Such emotions and values as happiness, mood, well being, "high", spiritual feeling, stress, etc. were rated. Other questions tapped demographic information (e.g., major, membership in musical groups) and amount of musical training. The SEES was completed prior to the commencement of singers' rehearsals and performances.

Diary. A 14-item "Self-Report Form" (SRF) was also adapted from Beck et al.<sup>4</sup> The SRF were completed as "diaries" after each rehearsal and performance. The SRF consisted of ratings on emotions experienced on the *particular* day when the singing occurred and salivary samples were taken. Thus, the questions referred to short-term emotions that were personally associated by subjects with singing on specific days during the study. Such emotions as relaxation, feeling high, mood, stress, and satisfaction were rated. Other questions tapped information about the time and nature of the singing rehearsal or performance session, and whether singers had health or psychological issues on the days when saliva samples were collected.

Hypotheses and Other Research Questions

The following hypotheses were posed for solo singers:

Hypothesis 1. After rehearsals and performances, S-IgA levels will increase as a proportion of total protein as measured by pre-post differences.

Hypothesis 2a. After rehearsals, cortisol levels will either decrease or show no change as a proportion of total protein as measured by pre-post differences. Hypothesis 2b. After performances, cortisol levels will increase as a proportion of total protein as measured by pre-post differences.

Hypothesis 3. Cortisol levels would decrease if singers had positive emotions related to their performance.

Hypothesis 4. Singers who report relatively positive emotions on the SEES and SRF will have relatively larger changes in S-IgA after singing.

Hypothesis 5. Singers who report chronic stress during singing will have diminished or down regulated changes in S-IgA.

#### Results

# Hypothesis 1

S-IgA rose significantly during performance. Table 1 shows means and standard deviations for pre- and post-singing measures of the standardized concentration of S-IgA: S-IgA ( $\mu$ g/ml)/Whole Protein ( $\mu$ g/ml). Percent increase for performance S-IgA was significantly greater than 0, t(9) = 3.125, p = .012, but there were no significant changes during rehearsals. The pre-performance mean of the standardized concentration of S-IgA was 1162, and the post-performance mean was 1365, an increase of 19%. By contrast, the pre-rehearsal mean was 1148, and the post-rehearsal mean was 1188, an increase of only 3.5%. The percent increase for S-IgA in rehearsal was not significant, t(9) = 1.596, p = .145. This result is consistent with previous findings for choirs where changes during performances were much higher than during rehearsals. It suggests that the demands of performance, in front of teachers and an audience of peers, friends and family is both more exhilarating and stressful than rehearsals in which self-satisfaction and self-criticism constitute the principal sources of pleasure and pressure.

Table 1

Mean and Standard Deviation (in Parentheses) of Pre- and Post-Singing Standardized

Salivary Immunoglobulin-A Concentration (S-IgA Concentration/Whole Protein

Concentration) and Cortisol for Rehearsals and Performance (N=10)

	S-IgA (μg/ml) / Whole Protein (μg/ml)		Cortisol (pg/ml)	
	Pre-Singing	Post-Singing	Pre-Singing	Post-Singing
Rehearsal	1148.28 (582.15)	1188.83 (547.98) <sup>a</sup>	206.38 (69.41)	190.44 (84.60)°
Performance	1162.80 (653.16)	1365.30 (784.49) <sup>b</sup>	242.59 (178.54)	278.77 (248.92) <sup>d</sup>

<sup>&</sup>lt;sup>a</sup>Percent increase not significant, p = .145

# Hypothesis 2

Table 1 also shows mean pre- and post-singing cortisol measures for rehearsals and performance. *Hypothesis 2a.* Cortisol levels decreased moderately in rehearsals from a pre-rehearsal mean of 206 pg/ml to a post-rehearsal mean of 190 pg/ml, a drop of about 8%. This percent change was not significant, t(9) = -0.051, p = .960. *Hypothesis 2b.* However, cortisol <u>increased moderately</u> in performances, rising from a preperformance mean of 242 pg/ml to a post-performance mean of 272 pg/ml, an increase of 12%, although this change was also not statistically significant, t(9) = 1.253, p = .246.

<sup>&</sup>lt;sup>b</sup>Percent increase significant, p = .012

<sup>°</sup>Percent change not significant, p = .960

<sup>&</sup>lt;sup>d</sup>Percent change not significant, p = .246

## Hypothesis 3

As hypothesized, when singers reported relatively high satisfaction with their performance, there was a significant percent decrease in cortisol levels after singing: Correlation of reported satisfaction with percent cortisol change was negative, r(60) = -.365, p = .004. In contrast to the findings about Hypothesis 2, which failed to show a significant change of cortisol level after singing, this finding suggests that short-term positive emotion implied by satisfaction with one's singing performance probably contributed to downward percent change of cortisol during singing.

## Hypothesis 4

Correlations were performed on singers' positive emotions as reported on the SEES with changes in S-IgA. Two positive emotions were significantly correlated with an upward percent change of S-IgA: feeling of "well-being" during singing was highly correlated with upward change of S-IgA, r(10) = .802, p = .005; feeling "high" during singing was also significantly correlated with an upward percent change in S-IgA, r(10) = .688, p = .028. These findings confirm previous results for choir singers.

## Hypothesis 5

Correlations were performed on singers' chronic negative emotions as reported on the SEES and SRF with changes in S-IgA. Agreement with the statement on the SEES that "singing gives me identity as an artist" was negatively correlated with percent change in S-IgA at performance. Thus, S-IgA had a significant downward percent change for singers who reported such professional involvement, r(10) = -.663, p = .028. When singers reported on the SRF that they were "usually stressed during singing," they also tended to have a downward percent change of S-IgA during both rehearsals and performances, r(10) = -.288, p = .025. These findings advance our understanding of the

effect of chronic stress on immuno-enhancement. Those singers who had a relatively professional orientation toward singing as an artistic career apparently considered their performance in a more serious perspective, one in which their performance was a higher stakes endeavor. While the total number of practices or hours of rehearsal per week for these singers might also have influenced immunological responses to performance, this study did not collect information on these behaviors.

## Regression analysis

As a result of our findings that both positive emotions and acute stress contributed to upward percent change in S-IgA, and positive emotions contributed to downward change in cortisol, step-wise regression analyses were conducted. The dependent variables were upward percent change in S-IgA and downward percent change in cortisol, respectively.

The best predictive model for upward change in S-IgA after performance contained two variables obtained from the SEES: a positive measure of "well-being" and a negative measure of "artistic identity." Table 2 shows the regression statistics for the prediction of performance S-IgA change: The second step of the hierarchical regression, which included both the well-being and artistic identity variables, was significant, p < 0.018. This analysis indicates that those singers who report long-term pleasure (well-being) from singing and lack of professional identity related to singing tended to show an upward percent change of S-IgA after performing.

#### Table 2

Summary of Hierarchical Regression Analysis for Variables Predicting Percent Change in S-IgA during Performance (N=10)

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Variable	B	SE B	β		
Step 1					
Well-being	392.041	174.694	.622*		
Step 2					
Well-being	210.870	151.798	.334		
Artistic identity	-277.418	108.450	616**		

Note.  $R^2 = .386$  for Step 1, p = .055;  $\Delta R^2 = .297$  for Step 2, p = .018

However, none of the SEES or SRF responses could significantly predict changes in S-IgA after rehearsal, nor could these variables predict cortisol changes after rehearsal or performance.

### Discussion

This study advances our understanding of the effects of singing on immunocompetence as marked by transient S-IgA production. First, we found that S-IgA mobilization is prevalent in solo singers as was previously observed in choirs. The solo singers' relatively small increases in S-IgA can be explained by their high baseline levels, which may be a function of young adulthood, when compared to the lower levels of S-IgA measured from the middle-aged participants of previous studies.<sup>4,6</sup>

Second, we found, as in choirs, upward changes of S-IgA during performance were much greater than in rehearsals. This is particularly important because it points to the social context of singing on immune response. Because individual singers usually rehearse in isolation, there is neither social pressure nor immediate negative feedback for

<sup>\*</sup>*p*<.10

<sup>\*\*</sup>p<.05

a poor practice session. When solo singers perform their pieces for peers and instructors, the audience context brings both pressure to achieve and potential shame and disappointment if goals are not met. This conclusion was also supported, albeit weakly, by mean increases in cortisol during performances, as opposed to the modest decreases in the rehearsal condition, although these changes were statistically non-significant.

Third, the present study confirmed previous research in demonstrating the effects of psychological states on both S-IgA and cortisol production. Solo singers' positive emotions about their singing, such as feelings of well-being, were associated with increases in S-IgA production. Moreover, positive emotion, engendered by satisfaction with performance, was associated with a decrease in cortisol, a physiological manifestation of stress.

Fourth, the findings showed that stress is clearly implicated in down-regulating what we must assume to be the normal upward mobilization of the immune system. Self-reports of chronic stress during singing were associated with decreases in S-IgA.

Finally, the correlation findings and regression analysis pointed to the presence of both a sense of well-being from singing as a positive factor and involvement with singing as a professional career as a negative factor on one's immune response to singing. In light of the small sample of this study, this finding must be regarded as tentative. Yet the data showed that singers who regarded singing as less important in their artistic identity, and who believed that singing contributed to their well-being, were more likely to have stronger upward percent changes in S-IgA. This is important, because music conservatories may need to be concerned with the amount of stress implicated in professional development. To reiterate, there is some evidence suggesting that singing is physically demanding and may be associated with URTI.<sup>10</sup> Fahlman and Engels<sup>11</sup>

reported that the secretion rate of S-IgA may be the most useful clinical biomarker to predict the incidence of URTI. Given that these authors postulate a strong relationship between secretion rate and IgA decreases, the latter measure, as used in the present study, may be used as a measure of the adequacy of the immune system in response to disease.

Our results may point to important factors associated with both short-term health issues and students' long-term decisions pursuing professional careers. While a delicate balancing act, undergraduate singing majors need to retain the joy that many of them experienced during their pre-professional tenure as amateur members of choirs and as solo performers. Professional music instruction undoubtedly needs to be rigorous and demanding, but not at the expense of taking the pleasure out of singing. If pleasure is associated with normal, and even accelerated, immune response, as this and other studies have shown, then it is vital to preserve positive emotions during singing, not only for maintaining the joys of singing, but also, perhaps, to enhance bodily defenses against health risks.

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