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Impact of Music Experience on the Vascular Endothelial Response to Singing

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Abstract

Background: Vascular endothelial function is closely related to brain health, especially in individuals with cardiovascular risk factors. In a randomized, crossover clinical trial (NCT04121741), we have previously shown that 30 minutes of singing improves microvascular endothelial function in older adults with coronary artery disease. Here, we report on exploratory analyses, including (1) changes in cortisol and cytokine levels and their impact on vascular endothelial function, and (2) the impact of personal music experience on vascular function. **Methods:** Participants had three study visits separated by 2-7 days, according to a randomized, researcher-blinded, crossover, controlled design: (1) a 30-min period of live singing with an in-person music therapist, (2) a 30-min period of singing along to an instructional video and (3) a 30-min rest (control). Primary outcomes included macrovascular endothelial function assessed by brachial artery flow-mediated dilation (BA FMD%) and microvascular function assessed by peripheral arterial tonometry [Framingham reactive hyperemia index (fRHI) and reactive hyperemia index (RHI)]. Exploratory outcomes included (log) changes in salivary cortisol and cytokine (IL-6, TNF-α, IL-1β, IL-8) levels. Participants were asked to complete the Brief Music Experience Questionnaire (BMEQ), a 53-item validated self-report questionnaire designed to measure an individual's overall experience with music. The BMEQ assesses how people perceive, react to, and engage with music in various aspects of their lives. **Results:** Sixty-five subjects (mean age 67.7 ± 6.6 years, 40% female) completed the study. Compared to those subjects completing the BMEQ (n=31), there were no significant differences in age, sex, race, or presence of diabetes mellitus, hypertension, high cholesterol, heart failure, chronic kidney disease, or chronic respiratory disease in subjects who did not complete the BMEQ (n=34). Total BMEQ score did not impact changes in BA FMD% (-3.49 ± 2.00, p=0.086), changes in fRHI (0.58 ± 0.93, p=0.535), or changes in RHI (0.73 ± 0.65, p=0.262). When we decompose the sum of squares based on intervention, sex, race, and age, the BMEQ score does not predict changes in vascular function measures. In cross-over analyses, there were no acute changes in salivary cortisol or cytokine levels with 30 minutes of singing compared to control. Changes in IL-8 were directly related to changes in microvascular endothelial function (0.470 ± 0.184, p=0.012 for RHI and 0.780 ± 0.248, p=0.002 for fRHI). Changes in TNF-α were inversely related to changes in fRHI (-0.547 ± 0.263, p=0.040). Changes in cortisol concentrations were not related to measures of vascular function. **Conclusions:** The beneficial changes in microvascular endothelial function are not modified by personal music experience in older subjects with known coronary artery disease. There were no changes in salivary cortisol or cytokine levels after 30 minutes of singing compared to control. However, changes in cytokines IL-8 and TNF-α were related to changes in microvascular endothelial function, substantiating the known impact of these cytokines on vascular function.

Keywords: singing; endothelial function; cortisol; elderly

Introduction

Cardiovascular disease (CVD), which includes coronary heart disease, stroke, and peripheral arterial disease, is the leading cause of death (in most developed countries) threatening the health of the elderly [1]. A hallmark of vascular disease and aging is dysfunction of endothelial cells – the single layer of cells that lines blood vessels and the heart and regulates exchanges between the blood and tissues. Vascular endothelial function is closely related to brain health, especially in individuals with cardiovascular risk factors. Endothelial dysfunction is implicated in the development and progression of various cerebrovascular diseases, including stroke and small vessel disease, and is a key feature in chronic conditions like atherosclerosis and hypertension [2]. Assessment of vascular endothelial function has proven to be a useful tool in translational research since vascular endothelial function strongly predicts cardiovascular events in patients with and without CVD [3]. For example, there is an 8-13% lower risk of CVD events per 1% increase in brachial artery flow-mediated dilation [4]. Furthermore, endothelial dysfunction (and CVD risk) can be improved by interventions (such as physical exercise) known to reduce CVD risk [5–7]. However, CVD in older adults is often complicated by age-related complexities, including multi-morbidity, polypharmacy, frailty, deconditioning, falls, disability, and other challenges, making participation in physical exercise difficult. In U.S. adults ages 65 and over, only 15.3% of males and just 10.8% of females meet national recommendations for physical activity [8]. Alternative forms of therapy to reduce CVD burden and improve brain health outcomes are needed in this aging population.

Music as a therapeutic is attractive for a variety of reasons including minimal risk to patients, ease of use, accessibility, and pervasiveness across culture. Most prior literature has examined the impact of music listening on health outcomes [9], including positive effects on mental health and psychological stress outcomes, as well as some favorable changes in basic vital signs such as heart rate, blood pressure, respiratory rate and heart rate variability [9,10]. Other music-based interventions show promise for improvements in cognition and motor function in people with Parkinsons' disease, epilepsy, or multiple sclerosis [11]. Singing, a more active intervention (than passive music listening), may be more likely to influence physiological signals. Singing can cause changes in neurotransmitters and hormones, including upregulation of oxytocin, immunoglobulin A, and endorphins, which improves immune function and mood [12]. Furthermore, the physiological demands of singing were found to be comparable with walking at a moderately brisk pace,[13] providing biological plausibility that the health benefits of singing may overlap with that of exercise. However, unlike traditional physical exercise, the impact of singing on vascular health, especially in persons with known atherosclerotic vascular disease, has not been extensively studied.

In a randomized, crossover, controlled clinical trial, we have previously shown that 30 minutes of singing improves microvascular endothelial function in older adults with atherosclerotic coronary artery disease (CAD) [14]. The estimated improvement in microvascular endothelial function in our singing video intervention arm of this clinical trial translates into an impressive 25% reduction in CVD risk[4,14]. Here, we report on secondary analyses to explore potential mechanisms for the health benefits of singing, including (1) acute changes in cortisol and cytokine levels and their impact on vascular endothelial function, and (2) the impact of personal music experience on vascular function.

Methods

General Study Design

Data was obtained from our previously completed clinical trial, Singing and Cardiovascular Health in Older Adults (NCT04121741) [14]. The detailed methodology describing this study has been outlined in the previously published paper [14]. In brief, sixty-five subjects, ages 55 to 79, with established atherosclerotic coronary artery disease participated in the study. Participants had the following three study visits, separated by 2-7 days, according to a randomized, investigator-blinded, crossover, control design: (1) a 30-minute period of live singing with an in-person music therapist, (2) a 30-minute period of singing along to an instructional video directed by a voice professor, and (3) a 30-minute rest (control). All interventions were conducted with the subject in a seated position. To improve the transparency and specificity of reporting music-based interventions, a set of specific reporting guidelines for music-based interventions was followed [15].

Singing (And Control) Interventions

A video series was specifically created and recorded for the purposes of this study. The video singing intervention included an instructional sing-along video displaying a voice professor playing the piano and directing an elderly student in singing. The 10-minute vocal warm-up video included semi-occluded vocal tract (SOVT) activities, lip trills, humming, pitch modulation and face and tongue exercises. Full details of the video can be found in our prior publication [14]. The subjects selected 2 songs to sing for 10 minutes each from four different music genres including Folk (*This Land is Your Land)*, Pop *(Hey Jude)*, Country *(Jolene)*, and Hymn *(Amazing Grace)* - varying in tempo, melodic contour, and rhythm to fill the full 10 minutes per song. Lyrics were displayed along the bottom of the video screen on a Microsoft surface laptop.

The live music intervention included a live in-person singing session with a board-certified music therapist, who alternated between the keyboard or guitar depending on the subjects' music selection. This session also included a 10-minute vocal warm-up followed by two songs at 10 minutes each. Song choices were selected by the subject from a multi-genre binder targeting adults ages 55–79 and including over 40 song choices [14].

During the control intervention, subjects had a 30-min period of rest, sitting upright as they would be for the singing intervention. During this rest period, they were not allowed to sleep, watch television, read, browse their smartphone, or listen to music.

Measures of Vascular Endothelial Function

Vascular function measurements were performed before and after each study visit using the protocol previously described [14]. Macrovascular endothelial function was assessed using brachial artery flow-mediated dilation (FMD), a non-invasive method that evaluates changes in arterial diameter in response to reactive hyperemia induced by a short period of arterial occlusion [3]. FMD was measured by trained sonographers, who were blinded to the crossover treatment, using high-resolution ultrasonography with a 7.5 – 13 MHz probe. The results of FMD are expressed as the percentage change in post-stimulus arterial diameter compared to baseline (FMD%).

Microvascular endothelial function was assessed by digital peripheral arterial tonometry (PAT) using Endo-PAT 2000 device (Itamar Medical, Israel). PAT is a non-invasive technique that measures changes in pulse wave amplitude in response to reactive hyperemia [16]. It is expressed as the reactive hyperemia index (RHI) and Framingham reactive hyperemia index (fRHI) [17]. PAT and FMD measurements were performed simultaneously during each study visit.

Salivary Cortisol and Cytokine Collection

To assess the effects of singing on objective measures of the stress response and the immune system, we measured salivary cortisol and cytokine levels including interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-8 (IL-8) [18,19]. The subjects were asked to provide approximately half a teaspoon of saliva into a specialized collection tube, before and after each 30-minute singing (and control) visit. Samples were sent to an outside laboratory (Salimetrics SalivaLab) to have cortisol and cytokine levels measured. Salivary cortisol was measured using the Salimetrics Salivary Cortisol Assay Kit (Cat. No. 1-3002), and the cytokine panel was assayed using a proprietary electrochemiluminescence method developed and validated

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for saliva by Salimetrics. The average coefficient of variation in these measurements exceeded the applicable National Institute of Health guidance for Enhancing Reproducibility through Rigor and Transparency. A test volume of 25 µL was used for each determination, with two determinations performed per sample and the mean value reported. Salivary cortisol and cytokine concentrations were measured in µg/dL for cortisol and pg/mL for IL-1β, IL-6, IL-8, and TNF-α. To prevent outliers from unduly influencing the results, values exceeding 2.5 standard deviations from the cohort mean were removed from final analysis.

Borg Rate of Perceived Exertion (Borg RPE)

Borg RPE is a self-reported, user-friendly tool scaled from 6 (no exertion at all) to 20 (maximal exertion) [20]. Subjects' effort and exertion were measured by the Borg RPE scale after each study visit to assess perceived level of exertion during singing (or control). The Borg RPE is the preferred method to assess intensity among those individuals who take medications that affect heart rate or pulse due to the scale's ability to capture exertion from central cardiovascular, respiratory, and nervous system functions [21].

Brief Music Experience Questionnaire (BMEQ)

Participants were asked to complete the previously validated brief music experience questionnaire (BMEQ), a 53-item validated self-report questionnaire designed to measure an individual's overall experience with music. The BMEQ assesses how people perceive, react to, and engage with music in various aspects of their lives. The questionnaire tests the following 6 aspects of music experience: (1) commitment to music (the centrality of pursuit of musical experiences in the person's life); (2) innovative musical aptitude (self-reports of musical performance ability as well as the ability to generate musical themes and works); (3) social uplift (the experience of being uplifted in a group-oriented manner by music); (4) affective reactions (affective and spiritual reactions to music); (5) positive psychotropic effects (calming, energizing, integrating reactions); and (6) reactive musical behavior (motile reactions including humming and swaying along with music) [22]. Respondents' choices on a five-point scale represent their level of agreement with survey statements from 1 (Very untrue) to 5 (Very true). Statements are intended to be relevant to non-musicians as well as musicians. Higher score on the BMEQ indicate a greater level of musical engagement and a more profound experience with music. This questionnaire was used with the permission of the authors.

Statistical Analysis

The Statistical Analysis System (SAS® 9.4) was used for all statistical analyses. Crossover trials with three treatment arms have a widely accepted analysis plan that we followed [23]. First, with three treatment arms, the carry-over effect can be estimated from one time-point to the next (something that is not possible with two treatment arms). If the p-value for the carry-over effect is >0.1, then no carry-over is considered further. There are up to four components that are estimated with unbalanced analysis of covariance by regression (fixed effects only, no random effects) in the primary analysis in this order: i) an intercept for each participant; ii) the timing of the treatment (first, second or third); iii) the treatment itself (singing with live coaching, singing along to instructional video, or control without singing); and iv) the carry-over effect, if any. With this base model, we added a term for the BMEQ for a secondary model but, obviously, this was limited to those who completed the BMEQ.

Because FMD and RHI/fRHI were measured before and after each treatment, we analyzed the absolute change (after-before). Similarly, cortisol and cytokines were measured before and after each treatment. However, cortisol and cytokines are concentrations that can vary by orders of magnitude between subjects, so we took natural logarithms (base e) before differencing, i.e., log_diff=log(after)-log(before).

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For FMD, RHI, fRHI, cortisol and cytokines, we identified outliers as >2.5 the study cohort's standard deviation. Generally, the number of outliers removed were few for each outcome. Additionally, we estimated FMD, RHI, and fRHI regression coefficients for cortisol and cytokines with crossover methodology in two ways: i) by removing outliers, and ii) rank normalization without removing outliers. If the p-values for the treatment arms were relatively close between these two models, then we concluded that assumption of normally distributed errors in method i) was accurate, and those results have been reported here. Because we have three primary outcome measures of vascular function, the significance level was adjusted to account for multiple comparisons using the Bonferroni correction, to minimize risk for type 1 error. The p-value for achieving statistical significance of our primary outcome was set at $p < 0.016$.

Results

Baseline characteristics are displayed in **Table 1.** Sixty-five subjects completed the study between January 07, 2020, and August 18, 2023. The mean age of participants was 67.7 (±6.6) years with 40% female. Most of the participants were non-Hispanic (98.5%) and identified as White (87.5%). The mean body mass index (BMI) of the participants was 30.0 (±8.3) with 49.2% categorized as obese and an additional 26.2% overweight. Physical limitation was ascertained by self-report with 53.8% reporting some level of orthopedic limitation. All participants had CAD, one of the mandatory inclusion criteria. History of MI was present in 63.1% of participants, coronary stenting in 74.6% and CABG in 27.7%. Among the comorbidities, dyslipidemia was most common and present in 84.6% of patients, followed by hypertension in 75.4%, and diabetes mellitus in another 29.2%. Forty percent of subjects were prior tobacco smokers with a mean cigarette pack year history of 15.5 ± 2.6 (SE).

Table 1. Baseline characteristics (n = 65).

	Total n=65(col%)	BMEQ n=31(col%)	No BMEQ n=34(col%)	p value
Age, mean ± SD, years	67.7 ± 6.6	68.4 ± 6.7	66.8 ± 6.5	0.373
Sex (n, % female)	26 (40.0)	16 (44.4)	10 (34.5)	0.415
Race				0.376
Black	7 (10.9)	3 (8.3)	4 (14.3)	
White	56 (87.5)	33 (91.7)	23 (82.1)	
Asian	1 (1.6)	0 (0.0)	1 (3.6)	
Unknown	1	0	1	
History of coronary artery disease				
Myocardial infarction	41 (63.1)	21 (58.3)	20 (69.0)	0.377
Coronary stent	47 (74.6)	21 (61.8)	26 (89.7)	0.011
Coronary artery bypass	18 (27.7)	12 (33.3)	6 (20.7)	0.258
Diabetes mellitus	19 (29.2)	9 (25.0)	10 (34.5)	0.403
Hypertension	49 (75.4)	28 (77.8)	21 (72.4)	0.618
High cholesterol	55 (84.6)	30 (83.3)	25 (86.2)	0.750
Chronic kidney disease	10 (15.4)	6 (16.7)	4 (13.8)	0.750
Chronic respiratory disease	18 (27.7)	12 (33.3)	6 (20.7)	0.258
Heart failure	12 (18.5)	6 (16.7)	6 (20.7)	0.678
Prior smoking	26 (40.0)	14 (38.9)	12 (41.4)	0.839
BMI, mean ± SD	30.0 ± 8.3	30.1 ± 6.9	29.8 ± 10.0	
BMI category				0.987
Underweight <18.5	4 (6.2)	2 (5.6)	2 (6.9)	
Healthy weight 18.5 - 24.9	12 (18.5)	7 (19.4)	5 (17.2)	
Overweight 25 to 29.9	17 (26.2)	9 (25.0)	8 (27.6)	
Obese 30 or greater	32 (49.2)	18 (50.0)	14 (48.3)	
Physical or orthopedic limitations	35 (53.8)	20 (55.6)	15 (51.7)	0.758
Level of limitation				0.277
None/Minimal	51 (78.5)	29 (80.6)	22 (75.9)	
Somewhat	12 (18.5)	7 (19.4)	5 (17.2)	
Very	2 (3.1)	0 (0.0)	2 (6.9)	

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In crossover analyses, there were no acute changes in salivary cortisol or cytokine concentrations with 30 minutes of singing. Crossover estimates between the two different singing interventions and control are reported as absolute change in (post- to pre-intervention) salivary (log) cortisol and (log) cytokine concentrations, **Table 2**. For change (±SE) in cortisol, the live music intervention showed an estimated difference of 0.04 ± 0.06 ($p = 0.471$), and the video intervention showed an estimated difference of -0.06 ± 0.06 ($p = 0.283$) compared control. For change in IL-1β, the live music intervention showed an estimated difference of -0.07 ± 0.10 ($p = 0.482$), and the video intervention showed an estimated difference of -0.04 ± 0.10 ($p = 0.715$) compared to control. For change in IL-6, the live music intervention showed an estimated difference of -0.17 ± 0.11 ($p = 0.121$), and the video intervention showed an estimated difference of -0.13 ± 0.11 ($p = 0.230$) compared to control. For change in IL-8, the live music intervention showed an estimated difference of -0.0018 ± 0.10 ($p = 0.986$), and the video intervention showed an estimated difference of 0.01 ± 0.10 ($p = 0.928$) compared to control. For change in TNF-α, the live music intervention showed an estimated difference of 0.01 ± 0.10 ($p = 0.943$), and the video intervention showed an estimated difference of 0.06 ± 0.10 ($p = 0.571$) compared to control.

Table 2. Salivary cortisol and cytokine outcomes comparing singing interventions to control.

	Absolute (post-pre)	
	Estimate (SE)	**p value**
Log cortisol		
Coach	-0.04 (0.06)	0.471
Video	-0.06 (0.06)	0.283
Log IL-1β		
Coach	-0.07 (0.10)	0.482
Video	-0.04 (0.10)	0.715
Log IL-6		
Coach	-0.17 (0.11)	0.121
Video	-0.13 (0.11)	0.230
Log IL-8		
Coach	-0.002 (0.10)	0.986
Video	0.01 (0.10)	0.928
Log TNF-α		
Coach	0.01 (0.10)	0.943
Video	0.06 (0.10)	0.571

*Adjusted for the order of the intervention, carry over and period. **Units for cortisol concentrations resulted in μg/dL; units for cytokine concentrations resulted in pg/mL.

Thirty-one subjects completed the BMEQ, **Table 1**. There were no differences in age, sex, race, or presence of diabetes mellitus, hypertension, high cholesterol, heart failure, chronic kidney disease, or chronic respiratory disease between those subjects completing the BMEQ (n=31) and those subjects not completing the BMEQ (n=31). Total BMEQ score did not influence changes in BA FMD% (-3.49 ± 2.00, $p = 0.086$), changes in fRHI (0.58 ± 0.93, $p=0.535$), or changes in RHI (0.73 ± 0.65, $p = 0.262$), **Table 3**. When we decompose the sum of squares based on intervention, sex, race, and age, the BMEQ score still does not predict changes in BA FMD%, RHI, or fRHI (p of 0.016 used to indicate significance). For absolute change in Borg RPE, the live music intervention showed an estimated difference of 4.33 ± 0.28 ($p < 0.0001$), and the video intervention showed an estimated difference of 3.65 ± 0.28 ($p < 0.00013$) compared to control. Compared to the video intervention, the live music intervention led to higher BORG RPE scores (9.98 ± 0.28 and 10.66 ± 0.29, respectively) with a difference of 0.68 ± 0.28), $p = 0.0167$. The BORG RPE was not a mediator or moderator of vascular function (results not shown).

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Table 3. Relationship between Brief Music Experience Questionnaire (BMEQ) score and vascular function.

	Absolute (Post - Pre)			
Parameter	**Estimate**	**SE**	**T value**	**p value**
The GLM procedure				
Brief MEQ – total score				
fRHI	0.58	0.93	0.630	0.535
RHI	0.73	0.65	1.130	0.262
BA FMD %	-3.49	2.01	-1.740	0.086
	Absolute (Post - Pre)			
Parameter	**Estimate**	**SE**	**T value**	**p value**
Type I SS				
Brief MEQ – total score				
fRHI	0.47	0.47	0.590	0.445
RHI	2.41	2.41	6.280	0.016
BA FMD %	3.53	3.53	0.730	0.397

*GLM is Generalized Linear Model. Type I SS is sum of squares.

The relationship between changes in cytokine concentrations and vascular function is displayed in **Table 4**. There is a direct relationship between change in IL-8 concentrations and change in fRHI, with an estimated difference of 0.78 ± 0.25 ($p = 0.002$). For TNF-α, there is a borderline significant inverse relationship with fRHI, with an estimated difference of –0.55 ± 0.26 ($p = 0.040$). There was no significant relationship of IL-1β or IL-6 with fRHI (-0.19 ± 0.26, $p = 0.455$ and 0.13 ± 0.14, $p = 0.381$, respectively). For RHI, there was a significant direct relationship with IL-8 (0.47 ± 0.18, $p = 0.012$). There was no significant relationship of IL-1β or IL-6 with RHI (-0.20 ± 0.19, $p = 0.302$ and 0.15 ± 0.11, $p = 0.155$, respectively). There was no significant relationship of TNF-α with RHI (estimated difference –0.26 ± 0.13, $p = 0.175$). The relationship between changes in cytokines and FMD were not significant (Supplementary Table 2a). The relationship between changes in cortisol levels and vascular function were not significant (Supplementary Table 1 and Supplementary Table 2b).

Table 4. Relationship between changes in cytokine concentrations and vascular function .

	Absolute (Post - Pre)			
	Estimate	**SE**	**T value**	**p value**
Framingham reactive hyperemia index (fRHI)				
Log IL-1β	-0.19	0.26	-0.750	0.455
Log IL-6	0.13	0.14	0.880	0.381
Log IL-8	0.78	0.25	3.150	0.002
Log TNF-α	-0.55	0.26	-2.080	0.040
	Absolute (Post - Pre)			
	Estimate	**SE**	**T Value**	**p value**
Reactive hyperemia index (RHI)				
Log IL-1β	-0.20	0.19	-1.040	0.302
Log IL-6	0.15	0.11	1.440	0.155
Log IL-8	0.47	0.18	2.560	0.012
Log TNF-α	-0.26	0.19	-1.370	0.175

*Units for cytokine concentrations resulted in pg/mL.

Discussion

In this secondary analysis of our clinical trial (NCT04121741), we investigated the effects of singing on salivary cortisol and cytokine levels, their relationship to vascular endothelial function, and the role of personal music experience. We used data from our previous randomized, crossover clinical trial, which demonstrated that 30 minutes of singing improves microvascular endothelial function (fRHI) in older adults with CAD [14].

No measures of micro- or macro-vascular function were significantly related to music experience, suggesting that the physiologic vascular benefits of singing may occur regardless of one's personal engagement and/or experience with music. This is an encouraging finding and aligns with the key principle that singing is a physical activity. In other words, you don't have to be a professional athlete or singer to reap the cardiovascular benefits of exercise or singing activities. This finding increases the attractiveness of singing in health promotion as an accessible, low-cost, scalable, safe and largely enjoyable intervention for a wide range of older adults, irrespective of prior music experience. While very few studies explore differences in health outcomes between professional and amateur singers, numerous studies - particularly on community singing groups and interventions with older adults - demonstrate mental (improved well-being,[12] decreased loneliness[24]) and physical (improved lung and cognitive[25] function) health benefits for participants across varying levels of singing ability [26,27].

In the present analysis, there were no significant changes in salivary cortisol levels after 30 minutes of singing compared to control. This may be related to the low intensity of the singing activities, as BORG RPE scores in the range achieved here (9.98 to 10.66) correspond to a low-intensity exercise. In general, moderate to high-intensity exercise causes a temporary increase in cortisol levels. However, the extent of the increase depends on the intensity, duration, and type of exercise [28]. Another possible explanation for the null cortisol finding could be that the parasympathetic activation with singing (i.e. through stimulation of the vagus nerve with vocal cord vibrations and innervation of the larynx and pharynx)[29] counterbalances the stress response of the singing (exercise) activity, helping to maintain cortisol levels and promote relaxation. In fact, a separate study in 32 older adults, mean age 64.3 years, singing just one song (mean singing duration 3 minutes and 50 seconds) resulted in a decrease in salivary cortisol levels [27]. Perhaps the short duration of the singing in this study (compared to 30 minutes in our singing interventions) was enough to stimulate the parasympathetic nervous system with little activation of the sympathetic nervous system, resulting in a reduction in cortisol levels.

There were no significant changes in salivary cytokine levels after 30 minutes of singing compared to control. This finding differs from a prior study of 193 subjects in populations affected by cancer (carers, bereaved carers, and patients) with mean age 56.9 years, showing increases in various cytokines (IL-2, IL-4, IFN-Ɣ, and TNF-α) after one hour of group singing in a choir format [30]. They also saw reductions in cortisol levels. It may be that the group singing format is more likely to modulate components of the immune system through improved mood, related to factors such as social cohesion and connectedness. Perhaps the longer duration of the intervention (1 hour) was needed to see meaningful changes in the cytokine levels. The main limitation, however, of this positive study is the lack of a control arm or group for comparison, reducing the rigor of the study conclusions. We also considered that singing might provoke anxiety for some people. Anxiety is typically associated with unfavorable changes in pro-inflammatory cytokines[31] compared to healthy controls and therefore, may negate any potential improvements in these markers if anxiety were not a contributing factor. Future research needs to consider the moderation or mediation of emotions (on health-related outcomes) interacting with singing. Singing in a group may lessen anxiety [32] associated with singing and would also be more scalable from a healthcare systems standpoint.

Exploratory analyses in our study did show a significant direct association between changes in IL-8 and microvascular function (both RHI and fRHI) whereby elevations in IL-8 correspond with improvements in microvascular endothelial function. IL-8 is known to play a complex role in vascular

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endothelial function, influencing both pro-angiogenic and even pro-inflammatory processes. It can promote endothelial cell migration, proliferation, and survival, and increase vascular permeability, all crucial aspects of angiogenesis (new blood vessel formation) but also potentially contributing to coronary plaque formation (pro-inflammatory) [33]. In this patient cohort with known CAD, angiogenesis is often part of the healing and recovery process. Angiogenesis leads to the development of collateral vessels in the heart muscle to improve blood flow to ischemic areas that are deprived of oxygen and nutrients. As part of this process, IL-8 stimulates endothelial cells to migrate and divide, which are essential steps in angiogenesis. Given our finding that IL-8 levels correspond to improvements in microvascular endothelial function in this study population, we may be able to surmise that angiogenesis (rather than pro-inflammation) is the predominant mechanism for this finding. With regards to brain health, IL-8 may have neuroprotective effects. For example, IL-8 levels in cerebrospinal fluid have been associated with better memory performance and cognitive improvement in cognitively healthy, older adults [34].

Additionally, salivary TNF-α was inversely associated with fRHI. This cytokine is typically considered pro-inflammatory and is released by immune cells, such as macrophages and T cells, in response to infection, injury or other inflammatory stimuli. In terms of vascular function, TNF-α mediated signaling initiates and accelerates atherogenesis, thrombosis, vascular remodeling, vascular inflammation, vascular oxidative stress and impaired nitro oxide bioavailability, all contributing to vascular dysfunction [35]. Therefore, our finding that lower levels of TNF-α are associated with improvements in microvascular endothelial function is substantiated.

To our knowledge, this is the first study to explore the interplay between singing, inflammatory markers, and vascular endothelial function in older adults with CAD. While several prior studies have reported cardiovascular benefits from music listening interventions,[9,36] the specific act of singing - an active and engaging musical behavior - has received comparatively less attention. Our findings contribute to a growing body of literature that suggests singing can have physiological benefits, at least in part mediated by improvements in microvascular endothelial function.

This study is not without limitations. As a secondary analysis of a clinical trial that was not powered for these mechanistic outcomes, the results should be interpreted with caution. The sample included only older adults with CAD, limiting generalizability to other populations. Furthermore, the study assessed only short-term effects; long-term vascular function outcomes of regular singing practice remain unknown. We also acknowledge the potential variability in cytokine responses, which may be influenced by factors such as time of day, recent physical activity, or acute stress levels [37]. However, our subjects completed all study visits with vascular function studies in the mornings and in the fasted state. Finally, only 31 subjects completed the BMEQ; therefore, we may be underpowered to detect a true relationship between music experience and the vascular function response to singing.

Despite these limitations, our findings offer preliminary evidence that a single 30-minute singing session can benefit vascular endothelial function and that these effects are unrelated to personal music experience. This has potential clinical and community implications, as singing is a low-cost, culturally adaptable activity that may support cardiovascular health in aging populations. Future studies should aim to replicate these findings in larger, more diverse cohorts and assess the longitudinal and sustained effects of repeated singing sessions on vascular function. Additionally, research should explore the mechanistic pathways linking singing to vascular outcomes, including autonomic regulation, inflammatory signaling, and psychosocial factors such as mood and social connectedness.

Conclusions

The beneficial changes in microvascular endothelial function are not modified by personal music experience. In older subjects with known coronary artery disease, there were no changes in salivary cortisol or cytokine levels after 30 minutes of singing compared to control. Future studies need to

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explore the sustained vascular response to singing over weeks to months and consider the group singing format as a more tangible intervention in healthcare settings.

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