Greater BOLD Response to Working Memory in Endurance-Trained Adults Revealed by Breath-Hold Calibration

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Abstract: *Background:* Cardiorespiratory fitness is associated with increased frontal and parietal activation during executive function tasks. While these findings suggest fitness-related enhancement of neuronal response, the utility of functional magnetic resonance imaging (fMRI) may be limited by potential fitness-related differences in global vascular reactivity. The aim of this study was to determine if highly fit adults display differential activation during working memory after calibration for vascular reactivity relative to their sedentary counterparts. *Methods:* Thirty-two endurance-trained and 24 sedentary adults, aged 40–65 years, completed a 2-Back verbal working memory task and a breath-hold challenge during fMRI. Group differences in blood oxygen level-dependent (BOLD) response during working memory were examined across the whole brain and in a priori regions of interest (ROI) before and after breath-hold calibration using non-parametric permutation testing. Multiple regression was used to explore the association between cardiorespiratory fitness (VO₂max), age, and calibrated 2-Back-related activation within the one a priori ROI with significant group effects. *Results:* In comparison to the endurance-trained group, the sedentary group exhibited greater BOLD signal changes in response to the breath-hold task. After, but not before calibration, the endurance-trained group displayed significantly higher 2-Back-related activation in the right middle frontal gyrus ($P = 0.049$). Older age predicted lower 2-Back-related activation ($\beta = -0.308$, $P = 0.031$), whereas fitness predicted higher activation ($\beta = 0.372$, $P = 0.021$) in this region. *Conclusions:* Breath-hold calibration increased detection of working memory-related BOLD response differences between sedentary and endurance-trained adults. Moreover, cardiorespiratory fitness appeared to mitigate age-related changes in BOLD during working memory in this region. *Hum Brain Mapp* 35:2898–2910, 2014. © 2013 Wiley Periodicals, Inc.

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INTRODUCTION

Cognitive abilities steadily decline across the latter decades of life, particularly within the domains of working memory, processing speed, and selective attention [Li et al., 2001]. Interestingly, the trajectory of decline is highly variable [Ylikoski et al., 1999], suggesting that genetic and lifestyle factors may attenuate cognitive aging. In particular, engagement in regular aerobic exercise is recognized for protecting cognitive ability [Barnes et al., 2003; Colcombe and Kramer, 2003]. Understanding the physiological mechanisms that underlie fitness-related cognitive benefit is crucial for the development of optimum interventions to ensure successful aging. Ideally, this work would incorporate highly sensitive measures to examine the earliest stages of vulnerability, which precede the onset of significant functional decline.

Over the past 2 decades, functional magnetic resonance imaging (fMRI) has emerged as a popular method for studying the brain in asymptomatic individuals at risk for future cognitive decline. Alterations in the blood oxygen level-dependent (BOLD) response have been reported in cognitively intact individuals at-risk for Alzheimer's disease and vascular dementia [Bookheimer et al., 2000; Braskie et al., 2010; Haley et al., 2007b] and interpreted as indicative of cognitive inefficiency and compensation. Similarly, fMRI has been utilized to study the beneficial effects of fitness on the brain. Older adults with greater cardiorespiratory fitness display superior performance and higher BOLD response in the frontal and parietal lobes during executive function tasks [Colcombe et al., 2004; Prakash et al., 2011]. These studies undoubtedly provide provocative evidence of the utility of fMRI for exploring the phenotypes associated with both risk factors for and protective factors against future cognitive decline. However, inferences from fMRI may be limited when it is expanded to populations outside of healthy young adults, as differences in vascular function may obscure interpretations from the BOLD signal.

The BOLD response is a composite signal of cerebral metabolic rate, cerebral blood volume, and cerebral blood flow [D'Esposito et al., 2003]. In healthy young individuals, neural response can be reasonably inferred from the BOLD signal since a close correlation between neuronal activity and the BOLD signal is well established [Heeger and Ress, 2002]. However, this relationship should not be automatically assumed for populations where differences in vascular functions are suspected [D'Esposito et al., 2003; Handwerker et al., 2007]. This includes studies on fitness since aerobic capacity has been associated with greater

vascular reactivity and compliance [DeSouza et al., 2000; Seals et al., 2008; Tanaka et al., 2000]. Fortunately, inferences from fMRI may be improved by implementing methods that reduce variability due to intra-individual differences in vascular function.

Recent studies on both young and older adults suggest that hypercapnia, secondary to a simple breath hold-task, can reduce vascular variability from the BOLD response [Handwerker et al., 2007; Riecker et al., 2003; Thomason and Glover, 2008; Thomason et al., 2007]. Hypercapnia induces a robust and diffuse increase in cerebral blood flow, which enhances the BOLD signal despite minimal cognitive engagement [Kastrup et al., 2001; Li et al., 1999]. Since hypercapnia and cognitive tasks produce similar hemodynamic responses [Bandettini and Wong, 1997; Cohen et al., 2004; Handwerker et al., 2007], a voxel-wise calibration approach can be used to reduce variance due to non-neural components of the BOLD signal. Thomason et al. [2007] found that a breath-hold calibration procedure decreased intersubject variability in the BOLD response to working memory by approximately 25%. Thus, a breath-hold calibration approach may provide greater sensitivity for detecting fitness-related neural differences in the BOLD response.

Accordingly, the aim of this study was to explore fitness-related changes in the BOLD response during cognition before and after breath-hold calibration in a population of healthy middle-aged adults. A cross-sectional approach was used to compare the BOLD response in sedentary and endurance-trained adults. Participants completed a hypercapnic challenge (breath-hold) as well as a 2-Back working memory task during fMRI. The 2-Back task was selected because it engages cognitive processes that show the greatest fitness-related benefit [Colcombe and Kramer, 2003]. The breath-hold data were modeled with a traditional boxcar design convolved with a canonical double gamma hemodynamic response function since this method is most broadly used in fMRI research and therefore has the widest applicability. Additionally, these results were supplemented by modeling with a sine-cosine wave at the task frequency, since hypercapnia paradigms with short breath-hold periods tend to be sinusoidal in nature [Murphy et al., 2011; Thomason and Glover, 2008]. Moreover, the onset of BOLD response during breath-hold is delayed by 10–15 s [Liu et al., 2002] and sine-cosine modeling enables a flexible approach to identify the peak-to-peak value in each voxel. Based on prior literature [Colcombe et al., 2004; Prakash et al., 2011], it was hypothesized that endurance-trained adults would demonstrate higher 2-Back-related activation in the frontal and

parietal cortices. Moreover, calibration by the hypercapnia task was anticipated to increase sensitivity for detecting cognitive-related BOLD differences, regardless of the approach used to model the breath-hold data.

MATERIALS AND METHODS

Sedentary and endurance-trained adults, ages 40–65 years, were recruited from the greater community. Participants were classified as endurance-trained if they reported engaging in moderate or strenuous aerobic exercise (running and/or cycling) at least 4 days per week on the International Physical Activity Questionnaire Short Form [Craig et al., 2003]. Participants were classified as sedentary if they reported no regular physical exercise in the past year. Maximal oxygen consumption was used to verify self-reported exercise training [Wilson and Tanaka, 2000]. The age range of the sample was restricted to middle-aged adults in alignment with the World Health Organization's recommendations for implementing early interventions [World Health Organization, 2005]. Eligibility criteria included a negative medical history for overt coronary artery disease, neurological disease (e.g., stroke, Parkinson's disease, clinically significant traumatic brain injury), major psychiatric illness (e.g., schizophrenia, bipolar disorder), and substance abuse (i.e., diagnosed abuse and/or previous hospitalization for substance abuse) as assessed by a medical history questionnaire. Additionally, current smoking, cardiovascular-acting medications, diabetes (fasting glucose > 126 mg/dl), left-handed dominance, impaired global cognitive function (Full Scale Intellectual Quotient < 85), and excessive movement in the scanner (translational displacement > 2.5 mm in any plane) were exclusionary criteria. Fifty-nine participants fulfilled the eligibility criteria and were included in the study sample upon providing written consent. Two participants were removed from the analyses for excessive movement in the scanner and one participant was removed for diabetes. The final sample was composed of 32 endurance-trained participants and 24 sedentary participants. Participants self-identified as the following: 83.3%-Caucasian, 5.6%-African American, 3.7%-Hispanic, 1.8%-Asian, and 5.6%-Other/Did Not Specify.

Procedures

This study was conducted in accordance with the Helsinki Declaration of 1975. The local Institutional Review Board approved the study and all participants provided written informed consent before enrollment. A medical history questionnaire was used to code medical conditions and treatments as either or present or absent based on participants' self-report. Participants completed three assessments (general health, cardiorespiratory fitness, and neuropsychological/brain imaging) on separate days and completed the study within 2 months.

General Health Assessment

Participants fasted for at least 12 h prior to the assessment. A physician's balance scale was used to measure body mass in kilograms and height in centimeters. Body mass index (BMI) was calculated by dividing body mass in kilograms by height in meters squared. Blood pressure was measured using a semi-automated device (VP-2000, Omron Healthcare, Bannockburn, IL) following 15 min of rest. Approximately 3 ml of blood was obtained from the antecubital vein by venipuncture. Standard enzymatic technique was used to assess fasting plasma concentrations of glucose and total-cholesterol.

Cardiorespiratory Fitness Assessment

Participants abstained from physical exercise for at least 24 h prior to the visit. A modified Bruce protocol of the graded treadmill exercise test was used to assess maximal oxygen consumption ($\text{VO}_{2\text{max}}$). After a 5-min warm up, participants ran or walked at an individually selected speed while the treadmill slope was raised 2% every 2 min until volitional exhaustion. Oxygen consumption (indirect calorimetry via respiratory gas measurements; Physio-Dyne, Quogue, NY), heart rate, and ratings of perceived exertion (the original Borg scale, [Borg, 1982]) were assessed throughout the protocol.

Neuropsychological/Brain Imaging Assessment

Neuropsychological assessment was conducted using standard clinical measures with established reliability and validity [Lezak et al., 2004]. In effort to reduce the number of comparisons, neuropsychological measures were categorized into one of three domains—global cognition, memory, or executive function. Domain scores were created by converting raw test scores into z-scores based on the study sample's mean and standard deviation. Timed tests were multiplied by -1 so that higher scores indicated better performance. Domain scores were computed for each participant by averaging the z-scores within the domain as follows: (1) *global*: Mini Mental Status Exam [Folstein et al., 1975] and Wechsler Test of Adult Reading [Wechsler, 2001]; (2) *memory*: California Verbal Learning Test II immediate recall, delayed recall, and recognition discrimination [Delis et al., 1987]; (3) *executive*: Trail Making Test Parts A and B time to completion [Reitan, 1958], Controlled Oral Word Associations Test [Ruff et al., 1996], and Wechsler Adult Intelligence Scale III Digit Span Subtest [Wechsler, 1997]. The neuropsychological test battery was administered and scored by a trained research assistant using standard administration and scoring criteria.

Imaging sessions consisted of task practice outside of the scanner, followed by T_1 -weighted imaging for anatomical reference, two imaging runs of the 2-Back task, and one imaging run of the breath-holding task. MRI data were acquired on a 3T GE Signa Excite scanner equipped

with a standard eight-channel head coil. An anatomical scan of the entire brain in the sagittal plane was collected using a high-resolution spoiled gradient echo sequence (256 × 256 matrix, flip angle = 15°, field of view (FOV) = 24 × 24 cm², 1 mm slice thickness, 0 gap). Functional imaging was performed during the 2-Back and breath-holding tasks = using a whole brain echo-planar imaging sequence (repetition time (TR) = 3,000 ms, Echo Time (TE) = 30 ms, flip angle = 90°, FOV = 24 × 24 cm², 64 × 64 matrix, 42 axial slices, 3 mm slice thickness, 0.3 mm gap). The functional tasks were presented with E-Prime software (Psychology Software Tools, Pittsburgh, PA), back-projected onto a screen positioned at the participant's head, and viewed through a double-mirror attached to the head coil.

Working memory was assessed with a verbal *n*-Back task consisting of 0-Back, 1-Back, and 2-Back blocks [Braver et al., 1997; Cohen et al., 1997]. During each block, a series of 15 individual consonants were visually presented in random order for 500 ms each with a 2,500 ms inter-stimulus interval. Participants made a response on a two-button MR-compatible response box to indicate whether or not the letter was a target (33% in each block). In the 0-Back condition, the target was a pre-specified letter (H). In the 1-Back and 2-Back conditions, the target was any letter identical to the one presented one or two stimuli before, respectively. Participants completed two consecutive 7-min runs consisting of three alternating blocks of 0-Back, 1-Back, and 2-Back conditions. Task performance was assessed for each condition by calculating mean accuracy and reaction time for correct trials.

A hypercapnic condition was induced using a simple breath-holding task adapted from Thomason and Glover [2008]. The task consisted of normal breathing, exhalation, and breath-hold blocks. The exhalation block was included since prior research has indicated that its inclusion reduces head movement at the initiation of the breath-holding condition [Handwerker et al., 2007]. Participants were visually cued by different colored squares to breathe normally (green square, 13.5 s), exhale (yellow square, 3 s), and hold their breath (red square, 13.5 s). Seven alternating blocks of each condition were repeated during a single imaging run lasting approximately 4 min.

Functional Imaging Analyses

Preprocessing

Functional data analysis was conducted using tools available from FSL v 4.1.2 (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). Preprocessing steps were identical for the 2-Back and hypercapnia tasks. Images were motion corrected using MCFLIRT [Jenkinson et al., 2002] and non-brain structures were removed using BET [Smith, 2002]. Images then underwent FILM prewhitening, high-pass filtering with a cut-off 100 s, and spatial smoothing with a 5 mm full width half maximum Gaussian kernel.

Hypercapnia task processing modeled with a Boxcar design

First-level analyses were performed with FSL's FEAT (FMRI Expert Analysis Tool, version 5.98) using the general linear model with regressors for block events (normal breathing, exhalation, breath-hold), motion parameters, and all temporal derivatives, following convolution with a canonical double-gamma hemodynamic response function. With FSL's registration tool, FLIRT, 7 degrees of freedom (DOF) were used to align each participants' functional image to his/her structural image and then 12 DOF were used to align the structural image to the Montreal Neurological Institute (MNI) 152 template [Jenkinson et al., 2002]. For whole brain analysis, the breath-hold > normal breathing contrast was explored with higher level mixed effects analyses using FSL's FLAME [Smith et al., 2004]. The Z (Gaussianized T/F) statistical images were thresholded by identifying clusters corresponding to $z > 2.3$ and applying a familywise error corrected cluster significance of $P = 0.05$. Details on a priori region of interest (ROI) analysis can be found further below.

Hypercapnia task processing modeled with a sine-cosine design

To enable a more flexible analytical approach, first level hypercapnia data were also modeled with sine and cosine waveforms with a duration equal to one cycle of the task (30 s) as well covariates for motion parameters and their temporal derivatives. Each participant's functional image was aligned to his/her structural image with 7 DOF and then each structural image was aligned the MNI 152 template with 12 DOF. Peak-to-peak estimates of the model fit were then calculated for each voxel by multiplying the sine wave regressors by their corresponding parameter estimates [(sine wave parameter estimate × sine wave time course) + (cosine wave parameter estimate × cosine wave time course)] to obtain predicted time courses and computing the peak-peak distance of the time course. For group analyses, each individual's peak-to-peak map was aligned with MNI space and then concatenated together in time. Whole brain analysis was performed using non-parametric permutation testing with the FSL Randomise tool. The rows of the design matrix were reordered 5,000 times and the maximum statistic across the brain for each sample was retained [Nichols and Holmes, 2002]. Clusters were identified using Threshold-Free Cluster Enhancement [Smith and Nichols, 2009] and applying a familywise error corrected cluster significance of $P = 0.01$. Details on a priori ROI analysis can be found below.

2-Back task processing

First-level analyses included block events (0-Back, 1-Back, 2-Back), reaction time for each trial, missed trials, motion parameters, and all temporal derivatives as

regressors, following convolution with a canonical double-gamma hemodynamic response function. Reaction time for each trial was included in the model since prolonged reaction time can confound cognitive-related signal activation [Honey et al., 2000]. Given the interest in examining working memory, 2-Back > 0-Back was the only examined contrast. FSL's registration tool, FLIRT, was used to spatially normalize the contrast estimates. 7 DOF were used to align each participants' functional image to his/her structural image and then 12 DOF were used to align the structural image to the MNI 152 template [Jenkinson et al., 2002]. Second-level analyses were performed to collapse the task-level contrast across runs using fixed effects. At the group level, the 2-Back > 0-Back contrast was explored with higher level mixed effects analyses using FSL's FLAME [Smith et al., 2004]. For whole brain analysis, the Z (Gaussianized T/F) statistical images were thresholded by identifying clusters corresponding to $z > 2.3$ and applying familywise error corrected cluster significance of $P = 0.05$. Details on a priori ROI analysis can be found below.

A priori ROI analysis

An a priori ROI analysis was performed on the 2-Back > 0-Back BOLD signal, the breath-hold > normal breathing BOLD signal derived from the boxcar design, and the absolute range of the peak-to-peak hypercapnia-induced BOLD signal obtained from sine-cosine design. To avoid circularity, eight a priori ROIs were created from published coordinates that were empirically derived from a verbal 2-Back task and have documented sensitivity for detecting alterations in relation to vascular factors [Haley et al., 2007b]. Given that the ROIs derived from Haley et al. [2007b] were reported in Talairach space [Talairach and Tournoux, 1988], GingerAle 2.0 (www.brainmap.org) was used to convert the coordinates into MNI coordinates [Laird et al., 2010]. The MNI coordinates were used to create 5 mm spheres and were then binarized in a mask. BOLD signal was extracted from each ROI for the three analyses (2-Back > 0-Back, breath-hold > normal breathing, and peak-to-peak hypercapnia response). BOLD signal values for the tasks modeled with a boxcar design (2-Back > 0-Back and breath-hold > normal breathing) were converted to percent signal change, as described in http://mumford.bol.ucla.edu/perchange_guide.pdf. In this calculation, the magnitude of the signal change was calculated by multiplying the parameter estimate of interest (2-Back for 2-Back task and breath-hold for the hypercapnia task) by a reference regressor height, dividing by the mean activation (over the entire time course), and then multiplying by 100.

Breath-hold calibration of the 2-Back task

To compare modeling approaches, the calibration procedure was performed separately with the boxcar design modeled hypercapnia data and sine-cosine modeled

hypercapnia data. For each of these analyses, a group level mixed effects design was employed with the 2-Back > 0-Back contrast as the dependent variable. For the hypercapnia data modeled with the boxcar design, the 4D file (fMRI data for the entire timeseries) created from the group level breath-hold > normal breathing analysis was included as a voxel-dependent regressor. For the hypercapnia data modeled with the sine-cosine design, the voxel dependent regressor was the absolute range of the peak-to-peak hypercapnia-induced BOLD. For both analyses, signal within the eight a priori ROIs was extracted from the residual functional data. Thus, for both approaches the calibrated 2-Back data represented the BOLD response for the task after the variance in breath-hold had been statistically removed.

Group analysis of a priori ROI

Group differences in the BOLD signal for the hypercapnia data modeled with the boxcar design, the hypercapnia data modeled with the sine-cosine design, and the 2-Back task before and after each calibration technique were explored using non-parametric permutation tests on the maximal T statistic. This method makes no assumptions about the shape of the distributions of the variables. Moreover, this method has been shown to preserve the type 1 error rate by applying the critical value for the maximum statistic over the ROI and produces similar outcomes as those derived from multiple comparisons correction based on random field theory with the general linear model [Nichols and Holmes, 2002]. Group labels were shuffled and 5,000 permutations of the Welch *t*-test were performed for each of the tests to create a null hypothesis distribution. *P*-values were determined from the max T distribution. Permutation analyses were performed in R [R Development Core Team, 2008], a freely available software package.

Statistical Analyses

Non-parametric chi-square or Mann-Whitney U tests were used to assess group differences in demographic and physiological variables since many of these variables have naturally skewed distributions. Group differences for the cognitive domain scores and task performance were assessed with ANOVA. As a follow-up analysis, multiple linear regression was used to explore the relationship between cardiorespiratory fitness (VO_2max) and calibrated 2-Back-related activation within the one a priori ROI with significant group effects, controlling for age and sex. Additionally, mean task accuracy and reaction time were correlated within the same ROI. A two-tailed α -level of 0.05 was used as the criterion of significant for follow-up analyses due to their exploratory nature. Statistical analyses described in this section were performed using SPSS 16.0 (SPSS, Chicago, IL).

TABLE I. Selected demographic & physiological characteristics

	Sedentary	Endurance-trained	P-value
Male/female	5/19	13/19	0.117
Education (years)	16.6 ± 1.8	17.3 ± 2.2	0.367
Age (years)	53.2 ± 5.3	51.4 ± 5.8	0.245
BMI (kg/m ²)	27.0 ± 6.5	23.2 ± 2.3	0.042
Systolic blood pressure (mm Hg)	118.2 ± 7.8	119.4 ± 13.2	0.993
Diastolic blood pressure (mm Hg)	71.8 ± 6.5	70.3 ± 7.4	0.461
Total cholesterol (mg/dl)	200.7 ± 29.9	191.1 ± 38.1	0.202
Blood glucose (mg/dl)	91.2 ± 8.3	89.2 ± 8.1	0.384
VO ₂ max (ml/kg/min)	25.3 ± 5.1	44.3 ± 8.8	<0.001

VO₂max = maximal oxygen consumption.

RESULTS

Descriptive Statistics

Group means and standard deviations for demographic and physiological variables are presented in Table I. The endurance-trained group had significantly higher cardiorespiratory fitness (VO₂max) and lower BMI than the sedentary group. No significant group differences were observed for sex distribution, age, years of education, systolic and diastolic blood pressure, total cholesterol, and fasting blood glucose concentrations. Cognitive domain z-scores and 2-Back task performance measures are presented in Table II. No significant group differences were observed for cognitive domain scores, but there was a trend toward better executive function performance and faster 0-Back reaction time in the endurance-trained group. Task performance was otherwise equivalent between groups.

Hypercapnia Activation Derived From the Boxcar Design

As seen in Table III, the breath-hold block yielded a negative percent signal change from baseline (normal breath-

TABLE II. Cognitive domain z-scores & 2-Back task performance

	Sedentary	Endurance-trained	P-value
Global	-0.007 ± 0.83	0.006 ± 0.67	0.948
Memory	-0.069 ± 1.11	0.052 ± 0.72	0.624
Executive function	-0.164 ± 0.61	0.123 ± 0.64	0.095
0-Back reaction time (ms)	784.9 ± 128.2	716.4 ± 127.4	0.052
2-Back reaction time (ms)	1197.3 ± 228.7	1132.4 ± 241.4	0.310
0-Back accuracy, % correct	96.1 ± 3.9	97.0 ± 4.9	0.474
2-Back accuracy, % correct	74.8 ± 8.7	77.8 ± 12.5	0.320

ing) in all examined ROIs. Breath-hold is usually associated with increased BOLD response but the onset of this effect is delayed by 10–15 s [Liu et al., 2002]. Thus, the expected peak would not occur until after the breath-hold block completed so negative values are anticipated. Activation significantly differed between the sedentary and endurance-trained groups in the left middle frontal gyrus with sedentary adults displaying a larger reduction in BOLD signal. The whole brain analysis of the individual and combined groups failed to detect any significant clusters of activation for the breath-hold > normal breathing contrast. However, the whole brain *t*-test comparing the groups displayed significantly higher (i.e., less negative) activation in the endurance-trained group in the right inferior temporal gyrus ($x = 42, y = -24, z = -26$), left rostral prefrontal cortex ($x = -34, y = 50, z = 8$), left precentral gyrus ($x = -28, y = -20, z = 40$), right thalamus ($x = 10, y = -32, z = 2$), left thalamus ($x = -6, y = -28, z = 4$), and left posterior cingulate gyrus ($x = -2, y = -38, z = 4$) (Fig. 1).

Hypercapnia Activation Derived From the Sine-Cosine Design

As seen in Table III, the task induced a large peak-to-peak BOLD response across the ROIs. As compared to the endurance-trained group, the sedentary group displayed

Figure 1.

Whole brain statistical image for breath-hold > normal breathing for the endurance-trained > sedentary group contrast (thresholded at familywise error corrected $P = 0.05$).

TABLE III. A priori ROI hypercapnia results for endurance-trained and sedentary groups

Brain region & MNI coordinates (X, Y, Z)	Percent change breath-hold > normal breathing	Peak-to-peak hypercapnia-induced BOLD signal
Left middle frontal gyrus -33, 8, 58	Sedentary: -1.06 ± 1.25 Endurance-trained: -0.64 ± 0.67 <i>P</i> -value: 0.369	Sedentary: 178.4 ± 116.4 Endurance-trained: 132.4 ± 80.4 <i>P</i> -value: 0.220
Left medial frontal gyrus -4, 24, 43	Sedentary: -0.71 ± 0.53 Endurance-trained: -0.59 ± 0.31 <i>P</i> -value: 0.615	Sedentary: 209.5 ± 77.3 Endurance-trained: 142.2 ± 53.7 <i>P</i> -value: 0.001 ^a
Right superior parietal lobule 40, -62, 59	Sedentary: -1.67 ± 2.31 Endurance-trained: -0.86 ± 1.15 <i>P</i> -value: 0.343	Sedentary: 137.5 ± 108.3 Endurance-trained: 119.2 ± 157.2 <i>P</i> -value: 0.783
Left inferior parietal lobule -50, -51, 49	Sedentary: -0.91 ± 1.08 Endurance-trained: -1.08 ± 0.98 <i>P</i> -value: 0.809	Sedentary: 151.4 ± 69.4 Endurance-trained: 175.0 ± 97.0 <i>P</i> -value: 0.498
Left middle frontal gyrus -45, 48, 8	Sedentary: -1.41 ± 0.77 Endurance-trained: -0.90 ± 0.60 <i>P</i> -value: 0.047 ^a	Sedentary: 227.7 ± 96.6 Endurance-trained: 148.6 ± 67.3 <i>P</i> -value: 0.002 ^a
Right superior frontal gyrus 36, 52, 9	Sedentary: -1.14 ± 0.58 Endurance-trained: -0.90 ± 0.49 <i>P</i> -value: 0.313	Sedentary: 258.0 ± 125.4 Endurance-trained: 184.7 ± 89.1 <i>P</i> -value: 0.040 ^a
Right middle frontal gyrus 35, 10, 56	Sedentary: -1.13 ± 1.20 Endurance-trained: -0.78 ± 0.84 <i>P</i> -value: 0.503	Sedentary: 171.6 ± 100.9 Endurance-trained: 141.0 ± 73.5 <i>P</i> -value: 0.392
Right inferior frontal gyrus 51, 16, -2	Sedentary: -0.92 ± 0.60 Endurance-trained: -0.85 ± 0.53 <i>P</i> -value: 0.850	Sedentary: 198.0 ± 69.2 Endurance-trained: 164.8 ± 77.9 <i>P</i> -value: 0.210

^a*P* < 0.05.*P*-values are derived from the group comparison permutation tests.

significantly greater peak-to-peak response in the left medial frontal gyrus, left middle frontal gyrus, and the right superior frontal gyrus. The whole brain analysis of the individual and combined groups indicated robust positive activation throughout the brain. The permutation-based *t*-test comparing the groups indicated that the sedentary group displayed significantly higher activation in multiple clusters throughout the brain including left medial prefrontal cortex ($x = -6, y = 30, z = 34$), right medial prefrontal cortex ($x = 6, y = 26, z = 34$), left middle frontal gyrus ($x = -34, y = 26, z = 32$), left thalamus ($x = -10, y = -20, z = 10$), and right thalamus ($x = 12, y = -10, z = 12$) (Fig. 2).

2-Back Activation

Whole brain analysis of the 2-Back > 0-Back contrast revealed increased signal activation in expected areas

including the left inferior frontal gyrus ($x = -44, y = 6, z = 30$), right middle frontal gyrus ($x = 30, y = 14, z = 46$), left middle frontal gyrus ($x = -30, y = 2, z = 52$), left insular cortex ($x = -30, y = 20, z = -6$), right inferior parietal lobule ($x = 40, y = -50, z = 34$), and left inferior parietal lobule ($x = -36, y = -54, z = 36$) [Braver et al., 1997; Cohen et al., 1997] (Fig. 3). There were no significant differences between the groups for the whole brain and ROI analyses (Table IV, Fig. 4).

2-Back Activation After Breath-Hold Calibration

As shown in Table IV, the endurance-trained group displayed significantly higher 2-Back-related activation in the right middle frontal gyrus after calibration regardless of the model used for breath-hold data analysis [*P* = 0.049 for calibration with breath-hold > normal breathing (Fig. 5), *P* = 0.039 for peak-to-peak hypercapnia-induced

Figure 2.

Whole brain statistical image for peak-to-peak hypercapnia-induced BOLD signal for the endurance-trained > sedentary group contrast (thresholded at familywise error corrected *P* = 0.01).

Figure 3.

Whole brain statistical image for 2-Back > 0-Back (thresholded at familywise error corrected $P = 0.05$).

BOLD signal (Fig. 6)]. There were no significant group differences in 2-Back-related activation for any other a priori ROIs after breath-hold calibration.

Calibrated 2-Back Activation in the Right Middle Frontal Gyrus and VO_2max

Multiple linear regression was used to explore the association between the breath-hold > normal breathing calibrated 2-Back-related activation in the right middle frontal gyrus and cardiorespiratory fitness, controlling for age and sex. The overall model successfully predicted 2-Back-related activation ($P = 0.002$) with significant effects for both age ($\beta = -0.308$, $P = 0.031$, 95% CI: -3.925 to -0.201) and VO_2max ($\beta = 0.372$, $P = 0.021$, 95% CI: 0.189 – 2.184). Sex did not account for any unique variance in the model ($\beta = -0.151$, $P = 0.351$, 95% CI: -37.851 to 13.695). As seen in Figure 7, older age predicted lower 2-Back-related activation in the right middle frontal gyrus, whereas, higher

VO_2max predicted higher 2-Back-related activation in this region (Fig. 8). The results of the multiple linear regression were similar when the peak-to-peak hypercapnia calibrated 2-Back data was employed (overall model: $P = 0.002$; age: $\beta = -0.314$, $P = 0.027$, 95% CI: -4.043 to -0.251 ; VO_2max : $\beta = 0.363$, $P = 0.024$, 95% CI: 0.164 – 2.195 ; Sex: $\beta = -0.156$, $P = 0.336$, 95% CI: -38.940 to 13.551). It is not surprising that VO_2max was a significant predictor of calibrated 2-Back activation in the right middle frontal gyrus given that the groups were stratified based on physical activity level.

Calibrated 2-Back-Related Activation in the Right Middle Frontal Gyrus and 2-Back Task Performance

Higher breath-hold > normal breathing calibrated 2-Back-related activation in the right middle frontal gyrus was associated with faster 0-Back reaction time ($r = -0.279$, $P = 0.037$). There was also an association with better 2-Back task accuracy ($r = 0.272$, $P = 0.042$), but removal of an outlier diminished the significance of this effect ($r = 0.221$, $P = 0.105$). The associations with 0-Back accuracy ($r = -0.207$, $P = 0.125$) or 2-Back reaction time ($r = -0.221$, $P = 0.101$) were modest and did not reach statistical significance. The results were similar when the peak-to-peak hypercapnia calibrated 2-Back data was employed (0-Back reaction time: $r = -0.274$, $P = 0.041$; 2-Back reaction time: $r = -0.258$, $P = 0.055$; 0-Back accuracy: $r = 0.188$, $P = 0.164$; 2-Back accuracy $r = 0.235$, $P = 0.085$).

DISCUSSION

To our knowledge, this is the first study to demonstrate that breath-hold calibration improves detection of fitness-related differences in the BOLD response to cognitive challenge. After, but not before breath-hold calibration, the endurance-trained adults in our study demonstrated greater working memory-related activation in the right middle frontal gyrus as compared with age-matched

Figure 4.

Mean percent signal change for 2-Back activation in the right middle frontal gyrus in the endurance-trained and sedentary groups (data are means $\pm$ standard errors).

TABLE IV. A priori ROI 2-Back > 0-Back results for endurance-trained and sedentary groups

Brain region & MNI coordinates (X, Y, Z)	Pre-calibrated	Calibrated with breath-hold > normal breathing	Calibrated with peak-to-peak hypercapnia-induced BOLD signal
Left middle frontal gyrus -33, 8, 58	Sedentary: 0.56 ± 0.33 Endurance-trained: 0.59 ± 0.50 <i>P</i> -value: 0.952	Sedentary: -1.29 ± 20.93 Endurance-trained: 3.23 ± 29.79 <i>P</i> -value: 0.818	Sedentary: -4.95 ± 19.63 Endurance-trained: 4.38 ± 31.19 <i>P</i> -value: 0.492
Left medial frontal gyrus -4, 24, 43	Sedentary: 0.40 ± 0.29 Endurance-trained: 0.34 ± 0.23 <i>P</i> -value: 0.762	Sedentary: 4.62 ± 32.84 Endur- ance-trained: -0.84 ± 26.77 <i>P</i> -value: 0.818	Sedentary: 1.26 ± 33.84 Endurance-trained: 1.11 ± 25.82 <i>P</i> -value: 0.995
Right superior parietal lobule 40, -62, 59	Sedentary: 0.74 ± 0.69 Endurance-trained: 0.56 ± 0.82 <i>P</i> -value: 0.724	Sedentary: 7.04 ± 40.77 Endurance-trained: 3.36 ± 42.05 <i>P</i> -value: 0.930	Sedentary: 5.38 ± 33.69 Endurance-trained: 1.20 ± 48.29 <i>P</i> -value: 0.911
Left inferior parietal lobule -50, -51, 49	Sedentary: 0.41 ± 0.39 Endurance-trained: 0.63 ± 0.48 <i>P</i> -value: 0.231	Sedentary: -8.03 ± 33.21 Endurance-trained: 9.94 ± 35.86 <i>P</i> -value: 0.209	Sedentary: -7.10 ± 33.64 Endurance-trained: 7.88 ± 33.50 <i>P</i> -value: 0.349
Left middle frontal gyrus -45, 48, 8	Sedentary: 0.53 ± 0.45 Endurance-trained: 0.46 ± 0.57 <i>P</i> -value: 0.875	Sedentary: 3.31 ± 33.81 Endur- ance-trained: -1.52 ± 42.16 <i>P</i> -value: 0.890	Sedentary: 1.83 ± 33.14 Endur- ance-trained: -0.13 ± 42.88 <i>P</i> -value: 0.960
Right superior frontal gyrus 36, 52, 9	Sedentary: 0.46 ± 0.35 Endurance-trained: 0.31 ± 0.34 <i>P</i> -value: 0.352	Sedentary: 9.11 ± 38.35 Endur- ance-trained: -6.48 ± 40.78 <i>P</i> -value: 0.420	Sedentary: 5.72 ± 39.32 Endur- ance-trained: -4.09 ± 39.29 <i>P</i> -value: 0.719
Right middle frontal gyrus 35, 10, 56	Sedentary: 0.47 ± 0.40 Endurance-trained: 0.75 ± 0.67 <i>P</i> -value: 0.194	Sedentary: -12.76 ± 28.88 Endurance-trained: 11.69 ± 40.58 <i>P</i> -value: 0.049 ^a	Sedentary: -13.64 ± 26.62 Endurance-trained: 11.37 ± 41.99 <i>P</i> -value: 0.039 ^a
Right inferior frontal gyrus 51, 16, -2	Sedentary: 0.25 ± 0.28 Endurance-trained: 0.17 ± 0.27 <i>P</i> -value: 0.614	Sedentary: 5.19 ± 27.21 Endur- ance-trained: -2.04 ± 25.06 <i>P</i> -value: 0.663	Sedentary: 4.83 ± 27.23 Endur- ance-trained: -1.80 ± 25.22 <i>P</i> -value: 0.717

^a*P* < 0.05.*P*-values are derived from the group comparison permutation tests.

sedentary controls. These results suggest fitness-related differences in the neural response to a cognitive challenge as BOLD changes were detected over and above global

differences in vascular function. Follow-up analyses revealed that older age was associated with lower calibrated 2-Back response in the right middle frontal gyrus.

Figure 5.

Greater mean breath-hold > normal breathing calibrated 2-Back activation in the right middle frontal gyrus in the endurance-trained group as compared with sedentary controls (data are means $\pm$ standard errors).

Figure 6.

Greater mean peak-to-peak hypercapnia calibrated 2-Back activation in the right middle frontal gyrus in the endurance-trained group as compared with sedentary controls (data are means $\pm$ standard errors).

Figure 7.

Association between age and breath-hold > normal breathing calibrated 2-Back activation in the right middle frontal gyrus.

However, even after controlling for age, cardiorespiratory fitness was related to greater activation, indicating that fitness may serve to attenuate the effects of aging on the brain.

While a wide variety of analytic methods have been explored for modeling data from breath-hold tasks, very little of this work has been translated into calibration procedures for cognitive task activation studies. In an effort to bridge the literature, this study compared the performance of two distinct approaches, breath-hold > normal breathing contrast derived from the traditional boxcar design and peak-to-peak signal activation obtained from a sine-cosine model, as calibration procedures for a 2-Back working memory task. The breath-hold > normal breathing contrast derived from the boxcar design yielded a negative percent change across all a priori ROIs. This finding was not unexpected as hypercapnia-induced global rise in the BOLD signal is expected to be delayed by 10–15 s due to the accumulation of carbon dioxide over time, the primary driver of BOLD changes in breath-hold tasks [Liu et al., 2002; Murphy et al., 2011]. The initial decline in cerebral blood flow is related, at least in part, to autonomic regulation in response to changes in intrathoracic pressure [Thomason et al., 2005]. Thus, initial negative BOLD responses from the breath-hold > normal breathing contrasts are predictable in short duration breath-hold tasks (13.5 s). Additionally, the hypercapnia data in this study were modeled with a sine-cosine design, which enables the peak response to be flexibly captured regardless of the onset time. Utilizing this approach, the expected hypercapnia-induced global increase in the BOLD signal was observed. The hypercapnia data derived from both approaches were then used to calibrate the working memory task. For the calibration procedure, the 2-Back-related activation was the

signal extracted after the variance from the hypercapnia response had been statistically removed. Importantly, this technique produced similar results regardless of the type of design used to model the hypercapnia data. Our results indicate that breath-hold calibration can reduce variance due to vascular function irrespective of the technique employed to model the hypercapnia data.

One unexpected finding in this study was that endurance-trained adults displayed an attenuated BOLD response to hypercapnia (i.e., less negative BOLD response with the boxcar design and a less positive BOLD response with the sine-cosine modeling). A more robust response to breath-hold was predicted based on prior studies demonstrating increased cerebral blood flow at rest and during hypercapnia in association with aerobic fitness [Brown et al., 2010; Swain et al., 2003]. One possibility is that the diminished BOLD signal may reflect a lower ratio of oxygenated-to-deoxygenated hemoglobin secondary to increased cerebral metabolism. Aerobic exercise training stimulates brain markers of mitochondrial biogenesis [Steiner et al., 2011] and enhances mitochondrial enzymes that catalyze cellular oxygen uptake in the brain [Navarro et al., 2004]. Thus, the oxygenated to deoxygenated hemoglobin ratio may be reduced in the endurance-trained group, attenuating the BOLD signal despite equivalent or elevated cerebral blood flow delivery. Alternatively, breath-hold may have induced a comparatively modest change in cerebral blood flow and/or velocity in the endurance-trained group, perhaps as a function of enhanced autonomic regulation [Monahan et al., 2000; Sloan et al., 2009]. While determination of the mechanisms that underlie group differences in the BOLD response to hypercapnia are outside of the scope of this study, the results indicate significant alterations in the BOLD signal

Figure 8.

Association between VO_2max and breath-hold > normal breathing calibrated 2-Back activation in the right middle frontal gyrus.

between endurance-trained and sedentary adults that operate independently of cognition. Thus, calibration procedures may be instrumental in reducing variability in the BOLD signal that may obscure findings of cognitive-related BOLD differences. Accordingly, 2-Back related differences only were observed between sedentary and endurance-trained groups after the breath-hold calibration.

After breath-hold calibration, the endurance-trained group displayed greater BOLD response in the right middle frontal gyrus during the verbal working memory task in conjunction with faster 0-Back reaction times. Increased BOLD response was observed after removing the shared variance with hypercapnia indicating that fitness likely has beneficial effects on the neural processes underlying cognition over and above global fitness-related changes in cerebrovascular reactivity. This finding is consistent with the prior literature, which documents that while young adults tend to exhibit left lateralized prefrontal activation during verbal tasks [Smith and Jonides, 1997], middle-aged and older adults commonly display bilateral activation, a phenomenon described as “hemispheric asymmetry reduction in older adults” [Cabeza, 2002]. Hemispheric asymmetry reduction appears to compensate for age-related declines in neural and metabolic efficiency, thus allowing older adults to maintain successful task performance [Cabeza et al., 2002; Mattay et al., 2006; Reuter-Lorenz et al., 2000]. Using a verbal *n*-Back task similar to ours, Mattay et al. [2006] reported that older adults displayed greater bilateral prefrontal cortex activation in situations where they performed as well as younger adults. Similarly, in this study greater right frontal activation correlated with faster reaction time in the 0-Back condition. Given that our models controlled for reaction time, the increased BOLD signal is unlikely to be attributed to longer response latencies on incorrect trials. Moreover, prior studies have also reported that higher cardiorespiratory fitness is associated with greater activation during challenging executive tasks, particularly within the frontal and parietal cortices [Colcombe et al., 2004; Prakash et al., 2011]. Overall, high levels of aerobic fitness appear to enhance cognitive performance by enabling a more flexible engagement of cognitive resources [Colcombe et al., 2004].

The underlying mechanisms governing fitness-related increases in neural flexibility in humans are still unknown. However, studies on aging indicate that increased dopaminergic activity may be a component. Dopamine D2 receptor availability declines with age and predicts poor performance on frontally mediated tasks [Volkow et al., 1998]. In contrast, aerobic exercise enhances the survival and function of dopaminergic neurons by stimulating trophic factors such as brain derived neurotrophic factor [Hattori et al., 1994; Neeper et al., 1996]. Thus, exercise-stimulated trophic factors may attenuate age-related disruptions in dopaminergic function, enhancing the ability of the prefrontal cortex to maintain representations and improving executive function [Robbins and Arnsten, 2009]. Future work on cardiorespira-

tory fitness and dopaminergic activity will be necessary to fully explore this hypothesis.

The limitations of this study must be considered when interpreting the results. Our study utilized a cross-sectional design, which cannot assess causation. It is possible that individuals who elect to exercise may have pre-existing differences, which could account for the alterations in the BOLD response and the trends toward better executive function performance. However, the equivalence of the endurance-trained and sedentary groups in terms of age, education, and global cognitive ability helps to minimize this possibility. Additionally, while the results are intriguing, it must be acknowledged that the overall effect of the breath-hold calibration was relatively small and only significant in one ROI. Another potential limitation was that the duration of the breath-hold period was relatively short and may have resulted in a less robust hypercapnic response than a task with longer breath-hold periods. Moreover, the breath-hold method of hypercapnia has disadvantages. It requires a limited amount of cognitive engagement and does not ensure a standard dosing for all participants. Fortunately, breath-hold induced hypercapnia has been found to produce very similar results to 5% carbon dioxide inhalation [Kastrup et al., 2001]. Moreover, assessing the BOLD signal during breath-holding is advantageous in the sense that it does not require extra equipment and has better spatial resolution than transcranial doppler and arterial spin labeling [Kastrup et al., 2001]. Nonetheless, future studies with multiple indices of hypercapnic-induced cerebral blood flow would be beneficial.

In summary, we found that a simple breath-hold task increased detection of BOLD differences during cognitive challenge in sedentary and endurance-trained adults. After breath-hold calibration, endurance-trained adults displayed higher right middle frontal gyrus activation during a verbal working memory task. Moreover, older age was associated with lower BOLD response in this region, but cardiorespiratory fitness predicted greater signal even controlling for age. Thus, fitness appeared to mitigate age-related changes in the BOLD response during working memory in the right middle frontal gyrus. While longitudinal studies are necessary, the results indicate that cardiorespiratory fitness may enhance neural response during cognitive processing, which may slow the trajectory of age-related cognitive decline.

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