ORIGINAL RESEARCH

Carotid Artery Vascular Mechanics Serve as Biomarkers of Cognitive Dysfunction in Aortic-Banded Miniature Swine That Can Be Treated With an Exercise Intervention

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***Background*—**Cognitive impairment in the setting of heart failure with preserved ejection fraction remains poorly understood. Using aortic-banded miniature swine displaying pathological features of human heart failure with preserved ejection fraction, we tested the hypothesis that increased carotid artery stiffness and altered carotid blood flow control are associated with impaired memory independent of decreased cardiac output. Furthermore, we hypothesized that chronic exercise prevents carotid artery vascular restructuring and preserves normal blood flow control and cognition in heart failure with preserved ejection fraction.

***Methods and Results*—**Yucatan pigs aged 8 months were divided into 3 groups: control (n=7), aortic-banded sedentary (n=7), and aortic-banded exercise trained (n=7). At 6 months following aortic-banded or control conditions, memory was evaluated using a spatial hole-board task. Carotid artery vascular mechanics and blood flow were assessed at rest, and blood flow control was examined during transient vena cava occlusion. Independent of decreased cardiac output, the aortic-banded group exhibited impaired memory that was associated with carotid artery vascular stiffening, elevated carotid artery vascular resistance, and exaggerated reductions in carotid artery blood flow during vena cava occlusion. Chronic exercise augmented memory scores, normalized blood flow control, and improved indices of carotid artery vascular stiffening. Indices of vascular stiffening were significantly correlated with average memory score.

***Conclusions*—**Carotid artery stiffness and altered vasomotor control correlate with impaired cognition independent of cardiac systolic dysfunction. Carotid artery vascular mechanics may serve as a biomarker for vascular cognitive impairment in heart failure with preserved ejection fraction. Chronic low-intensity exercise reduces vascular stiffening and improves cognition, highlighting the utility of exercise therapy for treating vascular cognitive impairment in heart failure with preserved ejection fraction. **(*J Am Heart Assoc*. 2016;5:e003248 doi: 10.1161/JAHA.116.003248)**

Key Words: carotid compliance • exercise training • experimental models heart failure • remodeling • vascular cognitive impairment

The term *cardiogenic dementia* describes the link between heart failure (HF) and cognitive dysfunction.[1,2] Left ventricular (LV) systolic and diastolic dysfunction both appear to mediate the effect of HF on cognition. In HF with reduced ejection fraction (HFrEF), it is thought that reduced cardiac output (CO) leading to decreased cerebral blood flow[3–5] and impaired autoregulation[6,7] results in cerebral ischemia and subsequent cognitive decline.[8,9] In contrast to HFrEF, the link between LV diastolic dysfunction (ie, HF with preserved ejection fraction [HFpEF]) and cognitive impairment (CI) remains unclear.[10–12] Under resting conditions, patients with HFpEF exhibit normal CO and ejection fraction (EF) values,[13,14] raising the question of whether the cerebral hypoperfusion hypothesis is valid for HFpEF patients. Among community-dwelling[12] and hospitalized patients with HFpEF,[11] the prevalence of cognitive dysfunction may be as high as 50% and explained, in part, by the severity of HF[10] as well as noncardiac comorbidities including aging,[12] chronic kidney disease, hypertension,[11] and impaired glycemic control.[15] Whereas standard HF therapies may attenuate the progression of cognitive decline in HFrEF,[16] pharmacological treatments for HFpEF have proven largely ineffective.[17] Limited evidence indicates that CI is a predictor of mortality at the

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initial diagnosis of HF[18] and in hospitalized HFpEF patients[11] and suggests the clinical relevance and importance of elucidating its contribution to developing disease.

In a joint position statement, the American Heart Association and the American Stroke Association indicated that carotid artery stiffness may serve as a therapeutic target for vascular CI.[19] Arterial stiffness is a common feature of HFpEF[20–23] that supports the idea that HFpEF is a total-body disease that is reflected in multiple organ systems, including peripheral vascular dysfunction, and is possibly mediated by a systemic inflammatory state.[24,25] Furthermore, carotid artery stiffness correlates with decreased cerebral blood flow[26] and cognitive dysfunction.[26,27] CO and EF remain normal under resting conditions in HFpEF, and deficits in cognition may manifest as the result of peripheral vascular dysfunction. Consequently, carotid artery stiffness may serve as a clinical biomarker and/or therapeutic target for CI in HFpEF in the absence of impaired resting systolic cardiac function.

Using an aortic-banded miniature swine model displaying key pathological features of human HFpEF, including diastolic dysfunction, impaired contractile reserve, pathological cardiac remodeling, and lung congestion,[28,29] we tested the hypotheses that increased carotid artery stiffness and altered control of carotid artery blood flow are associated with impaired spatial memory independent of decreased resting CO. Given the reported benefits of exercise training on indices of arterial stiffness[26,30] and cognition,[26,31] we also tested the hypothesis that a chronic low-intensity interval exercise intervention prevents carotid artery vascular restructuring and preserves normal blood flow control and cognition in an experimental setting of HFpEF.

Methods

Study Design

Sexually mature intact male Yucatan miniature swine (aged 8 months; N=21) were separated into 3 groups: (1) nonsham sedentary control (CON; n=7); (2) aortic-banded sedentary (AB; n=7) and (3) aortic-banded exercise trained (AB-EX; n=7). At 2 months after surgery, aortic banding significantly increased LV diastolic wall thickness ($P<0.05$; 5.8±0.5, 7.5±0.7, and 8.3±0.4 mm for CON, AB, and AB-EX, respectively) but did not alter LV end-diastolic dimension (P value not significant; 46.1±0.7, 44.3±1.3, and 43.9±1.4 mm for CON, AB, and AB-EX, respectively), indicating that concentric LV hypertrophy was present prior to the onset of exercise training. At 5 months after aortic banding, cognitive testing began and continued for 3 weeks. At 1 week following the end of cognitive testing, pigs were anesthetized and carotid artery vascular mechanics and blood flow control were examined. Experimenters were not blinded during the collection of cardiovascular outcome measures. All animal protocols were in accordance with the "Principles for the Utilization and Care of Vertebrate Animals Used in Testing Research and Training" and approved by the University of Missouri animal care and use committee.

Aortic Banding

Aortic banding was performed, as described previously.[28] A 70-mm Hg trans-stenotic pressure gradient (74±2 and 74±1 for AB and AB-EX, respectively; P value not significant) was set under hemodynamically consistent conditions, defined as a peripheral vascular mean arterial pressure (MAP) of ≈90 mm Hg (90±1 and 91±1 for AB and AB-EX, respectively; P value not significant) set using phenylephrine (1–3 mg·kg·min^{-1} IV) at a heart rate of ≈100 beats/min (103±5 and 99±8 for AB and AB-EX, respectively; P value not significant).

Exercise Intervention

At 2 months following aortic banding, pigs in the AB-EX group began exercise training 3 days per week for 18 weeks. Exercise consisted of low-intensity interval running performed on a motorized treadmill.[28] Over the initial 4 weeks, pigs increased their workload gradually until each session consisted of (1) a 5-minute warmup at 2 miles per hour; (2) six 5-minute intervals at 3 miles per hour, with each 5-minute interval interspersed with a 3-minute interval at 4 miles per hour; and (3) a 5-minute cool-down period at 2 miles per hour.

In Vivo Cardiovascular Function

At 6 months following aortic banding, pigs were initially anesthetized using a mixture of Telazol (5 mg·kg^{-1}; Zoetis) and xylazine (2.25 mg·kg^{-1}) maintained using propofol (6–15 mg·kg·min^{-1} IV). At this dose, propofol does not impair cerebral autoregulation in pigs.[32] Pigs were placed in the supine position, and heart rate (ECG), body temperature (rectal probe), and MAP (fluid-filled 6F arterial catheter) were monitored continuously. Total peripheral resistance was calculated as quotient of MAP relative to CO. Carotid artery vascular mechanics (≈5 cm inferior to the carotid artery bifurcation) and blood flow (≈2 cm inferior to the carotid artery bifurcation) were measured using Doppler ultrasound (Phillips HDI 5000). Data for vascular mechanics were averaged over 3 cardiac cycles, and data for blood flow were averaged over 10 cardiac cycles at baseline and 3 cardiac cycles during transient vena cava occlusion. Local mechanical properties of the carotid artery were calculated, as described previously.[33]

Resting baseline carotid blood flow was calculated with a closed chest (ie, prior to the median sternotomy used to

perform pressure–volume loops) and measured as the product of the measured blood flow velocity and cross-sectional area of the carotid artery, as determined from the measured diameter. Shear rate was calculated as the product of 8 and the blood flow velocity, relative to the diameter of the carotid artery.[34] Carotid artery vascular resistance was calculated as the quotient of MAP and carotid artery blood flow. Resistive index was calculated as the difference between peak systolic velocity and end-diastolic velocity relative to the peak systolic velocity.

Pressure–volume loops were collected, as described previously.[28] A median sternotomy was performed, and a 5F admittance-based ADVantage catheter (Transonic Systems, Inc) was inserted into the left ventricle through a small apical incision. A 14F balloon occlusion catheter was inserted in the femoral vein and advanced to the inferior vena cava, and transient vena cava occlusion was achieved by inflating the balloon, during which time CO and peripheral MAP were monitored. Carotid artery blood flow was recorded at baseline, 5 seconds later, and at the end of vena cava occlusion. Cranial autoregulation was defined as the rate of regulation[35] during transient vena cava occlusion and calculated as the rate of change in carotid artery vascular resistance relative to the change in MAP from baseline to 5 seconds. According to this definition, a rate of regulation of $5\%\cdot s^{-1}$ indicates a per-second adjustment of 5% of the change necessary to compensate fully for changes in MAP.[35] We were able to perform comprehensive hemodynamic, ultrasound, and pressure–volume loop analyses of 5 to 7 animals per group, and those are reported in each table for individual measures.

Cognitive Testing

Spatial hole-board recognition tasks (modified from Bolhuis et al[36]) were performed in a 2×6-m testing arena with 10 bowls placed in the form of a "U." The entrance to the area was at the top of the U. The center of each bowl was 40 cm apart from the neighboring bowls. Three of the 10 bowls were baited with standard chow. To prevent visual cues, the bowls had removable lids, and the pigs were unable to see inside them. The pigs were able to open the lids with their snouts to gain access to the contents of the bowls. To prevent odor cues, all bowls contained a perforated grid with food placed above the grid in the 3 baited bowls and below the grid in the remaining 7 bowls.

After familiarization and standardization of trial performance among groups, exposure to the hole-board task was divided into 2 phases: (1) the acquisition phase and (2) the retention phase. The acquisition phase consisted of daily exposure to the spatial hole-board task for one 180-second trial (or until all food baits were located) for 5 consecutive days. The retention phase occurred 5 days following the last day of the acquisition phase and consisted of one 180-second exposure to the spatial hole-board task.

Scores for working memory (a form of short-term memory) were calculated as the ratio of the number of first-time visits to baited bowls to the total number of visits to baited bowls. Scores for reference memory (a form of long-term memory) were calculated as the ratio of the number of visits to the baited bowls to the total number of visits to all bowls.[37] Scores were measured on all 5 days of the acquisition phase and during the retention phase. Average memory score was calculated as the mean of working and reference memory scores during the retention trial. To enhance motivation, pigs were fasted overnight, and testing took place between 6 and 8:30 AM in the fasted state. Between trials, the testing arena and the spatial hole-board task were washed with 30% ethanol. The order of testing was randomized, and experimenters were blinded to the experimental group during testing.

Statistics

Baseline vascular characteristics and differences in memory scores for the retention phase were compared using 1-way ANOVA. Hemodynamic variables during transient vena cava occlusion and memory scores during the acquisition phase were compared using mixed-model ANOVA (group×time and trail, respectively). The location of significant interactions was determined using a post hoc Student Newman–Keul test. The Pearson correlation coefficient was calculated comparing carotid artery vascular mechanics versus average memory score for the retention phase. All data are reported as mean±SE, and significance is reported at $P<0.05$ levels.

Results

Cognitive Function

For the retention trial, working (short-term) memory scores were lower in AB versus CON pigs (Figure 1A). Working and reference (long-term) memory scores were greater in the AB-EX group compared with all other groups (Figure 1A and 1B).

Baseline Physical and Hemodynamic Characteristics

Body mass, EF, systolic and diastolic blood pressures, MAP, pulse pressure, and systolic and diastolic carotid artery diameters were similar between groups, and total peripheral resistance was increased in the AB group versus all other groups (Tables 1 and 2). Postmortem left ventricle plus

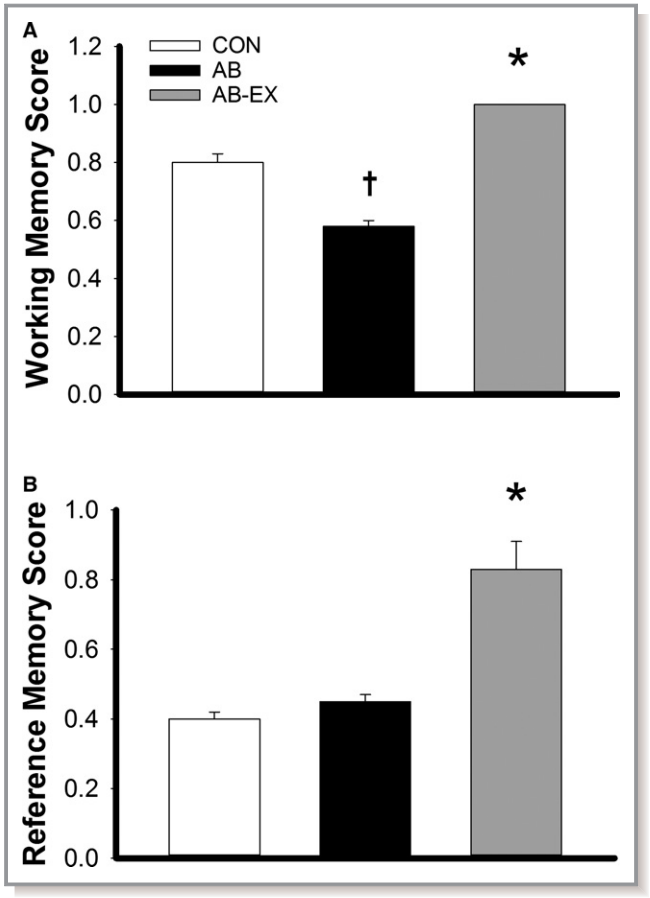

Figure 1. Working (A) and reference (B) memory scores during the retention trial phase. *P<0.05 vs all other groups; †P<0.05 AB vs CON. AB indicates the aortic-banded sedentary group; AB-EX, aortic-banded exercise-trained group; CON, nonsham sedentary control group.

septum mass, ratio of left ventricle plus septum to end diastolic volume, and end-diastolic pressure–volume relationship were all significantly increased in the AB and AB-EX groups versus CON (Table 2) and were observed in parallel to a strong trend toward increased diastolic septum wall thickness, indicative of concentric hypertrophy and impaired diastolic function. The Δ carotid artery diameter during the cardiac cycle was decreased in AB versus all other groups (Figure 2A). Carotid artery strain was reduced, and the Peterson elastic modulus was greater in AB versus all other groups (Figure 2B and 2C). Carotid artery stiffness was decreased, whereas distensibility and compliance were greater in AB-EX versus all other groups (Figure 2D through 2F). There was a trend toward decreased compliance in the AB versus CON group (P=0.09). Carotid artery blood flow and shear rate were reduced, and vascular resistance was greater in AB versus all other groups (Figure 3A through 3C). Resistive index was decreased in AB-EX versus all other groups.

Table 1. Physical Characteristics and Peripheral Vascular Hemodynamics

	Control (n=6)	Aortic-Banded Sedentary (n=6)	Aortic-Banded Exercise Trained (n=6)
Body mass, kg	46±3	48±2	49±3
Systolic blood pressure, mm Hg	82±7	103±8*	82±6
Diastolic blood pressure, mm Hg	61±8	80±5	67±5
Mean arterial pressure, mm Hg	68±8	88±6	72±6
Pulse pressure, mm Hg	21±2	23±5	15±2
Total peripheral resistance, mm Hg·L·min^{-1}	7.6±0.6	13.2±1.3†	9.6±1.1
Systolic carotid artery diameter, mm	5.8±0.3	5.8±0.2	5.8±0.1
Diastolic carotid artery diameter, mm	5.4±0.3	5.5±0.2	5.4±0.1

*P=0.09 vs control.
†P<0.05 vs all other groups.

Hemodynamics During Vena Cava Occlusion

CO was not significantly different between groups and decreased during vena cava occlusion in all pigs (Table 3). MAP was greater in the AB and AB-EX groups versus CON at baseline and at 5 seconds into vena cava occlusion but was similar between groups at the end of occlusion. Carotid artery blood flow was greater in AB-EX versus all other groups before and 5 seconds into vena cava occlusion; however, blood flow values were similar between groups at the end of occlusion (Table 3). There was a main effect of group for carotid artery vascular resistance, whereby vascular resistance was greater in the AB group versus all other groups and was lower in the AB-EX versus CON group (Table 3). The %Δ in CO decreased to a greater degree in the CON and AB groups versus AB-EX at 5 seconds, but the %Δ in MAP during vena cava occlusion did not differ between groups (Figure 4A and 4B). The %Δ in carotid artery blood flow decreased to a greater extent 5 seconds into vena cava occlusion in AB versus all other groups (Figure 4C). During vena cava occlusion, there was a main effect of group in which vascular resistance was greater at a given MAP in the AB group versus all other groups and was lower in the AB-EX versus CON group (Figure 4D). Furthermore, the rate of change in vascular resistance relative to MAP (ie, rate of regulation) was decreased in the AB group versus all other groups (Figure 4E).

Linear Regressions

All indices of carotid artery vascular mechanics were significantly correlated to average memory score

Table 2. ECG, Pressure–Volume Loop, and Postmortem Measures of Cardiac Parameters

	Control (n=5–7)	Aortic-Banded Sedentary (n=5–7)	Aortic-Banded Exercise Trained (n=5–7)
Ejection fraction, %	64±3	66±3	65±4
Septum wall thickness, mm	10.8±0.4	12.1±0.3*	11.8±0.5*
Left ventricle plus septum mass, g	140±6	171±6†	186±10†
End-diastolic volume, mL	105±11	103±9	103±7
Left ventricle plus septum to end-diastolic volume ratio	1.3±0.1	1.8±0.2†	1.7±0.1†
End-diastolic pressure–volume relationship, mm Hg/mL	0.005±0.001	0.011±0.002†	0.014±4†

*P=0.078 vs control.
†P<0.05 vs control.

during the retention phase. In addition, there was a significant negative correlation between carotid artery vascular resistance and average memory performance (Figure 5).

Discussion

The current study examined whether the cognitive dysfunction often observed in HF is dependent on a decrease in CO and

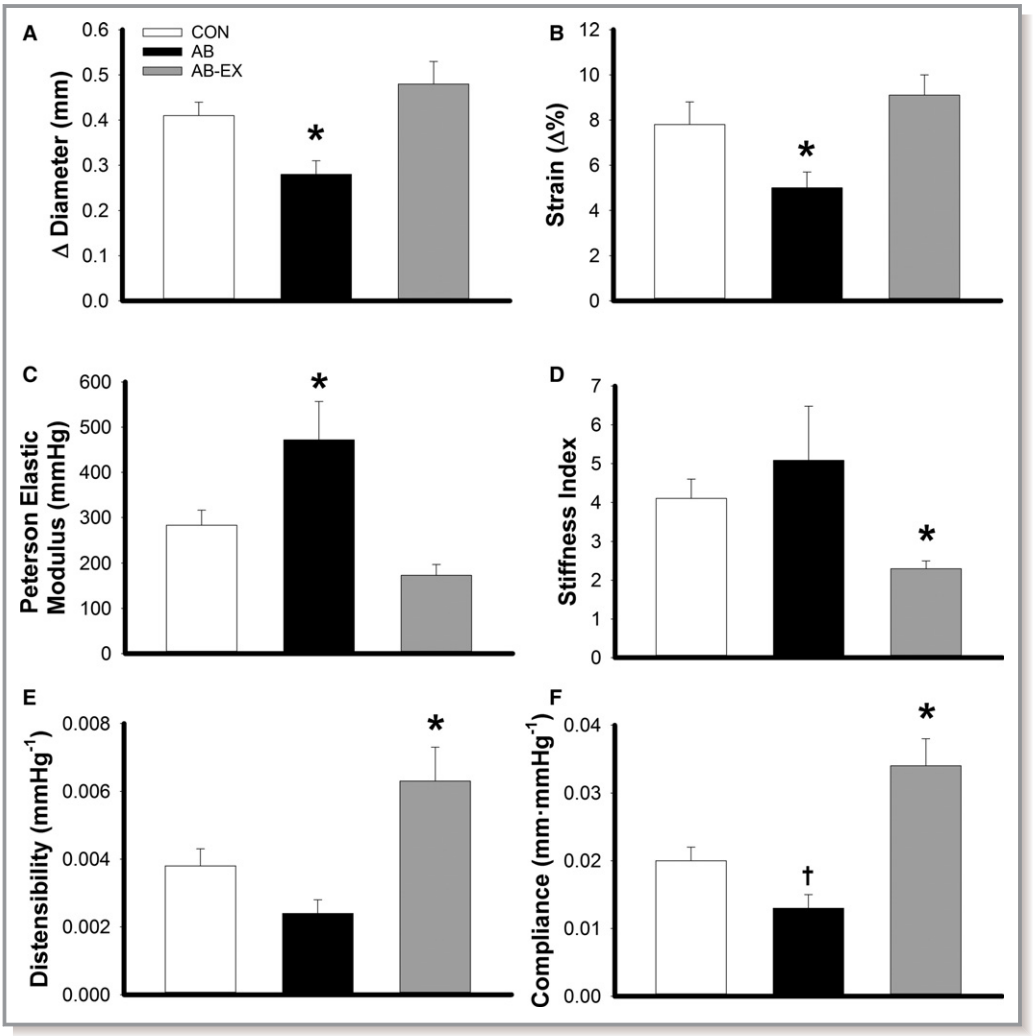

Figure 2. Carotid artery (A) Δ in diameter (mm), (B) strain (Δ%), (C) Peterson's elastic modulus (mmHg), (D) stiffness index, (E) distensibility ($mmHg^{-1}$) and (F) compliance ($mm{\cdot}mmHg^{-1}$) at rest.

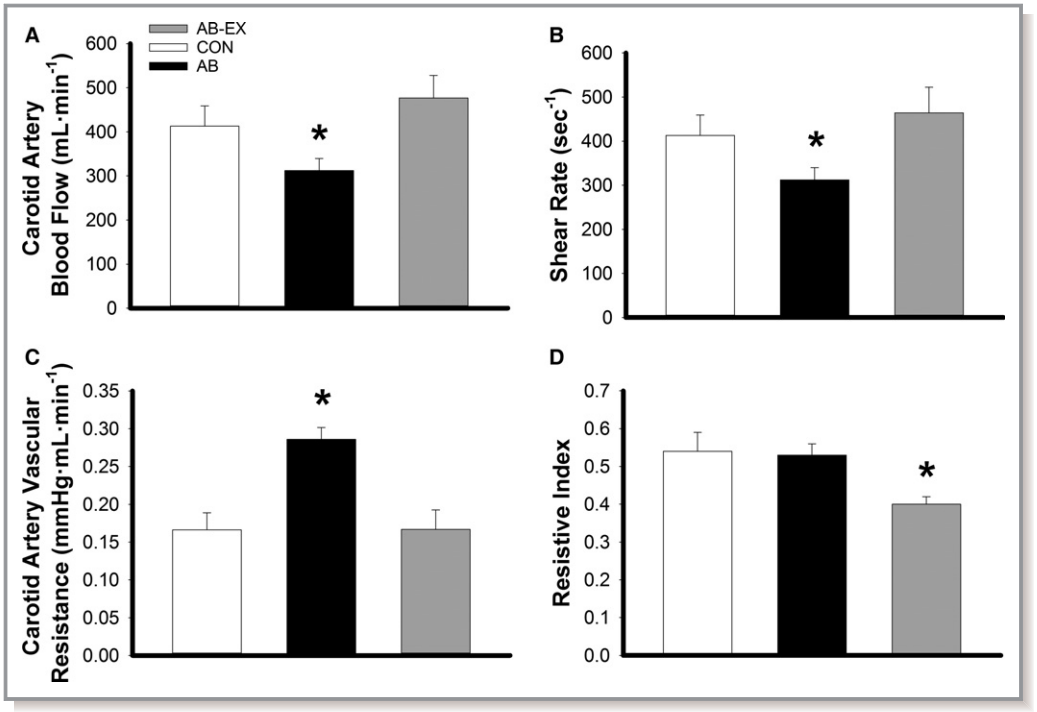

Figure 3. Carotid artery (A) blood flow (mL·min^{-1}), (B) shear rate (sec^{-1}), (C) vascular resistance (mmHg·mL·min^{-1}) and (D) resistive index at rest.

assessed the therapeutic effect of exercise training on this process in aortic-banded miniature swine. Our results revealed a number of novel findings. First, CI was evident in the AB group in the absence of a decline in resting systolic function (ie, resting EF percentage and CO were normal), implying that HF-induced dementia is not necessarily dependent on decreased CO. Second, cognitive dysfunction was strongly associated with increased peripheral vascular resistance, carotid artery stiffening, and impaired cranial blood flow responses during vena cava occlusion. Third, chronic low-intensity interval exercise training prevented cognitive and peripheral vascular dysfunction and improved carotid artery compliance despite chronic pressure overload at the level of the heart.

Cognition and Myocardial/Peripheral Vascular Function

The most interesting finding of the present study was that CI occurred independent of decreases in EF percentage or CO in AB animals. Carotid artery vascular stiffening, a characteristic often considered part of the HFpEF syndrome,[21,22] occurred in conjunction with deficits in working memory, suggesting that carotid stiffness coincides with or predisposes patients with HFpEF to CI. Independent studies in human HFpEF patients reported carotid artery vascular stiffening[21,22] and deficits in cognition.[10–12] The current study furthers this previous work by unifying these separate observations in a translational large animal model reminiscent of human HFpEF. The AB group displayed lower cranial blood flow, elevated cranial vascular resistance, and exaggerated %Δ in carotid artery blood flow during vena cava occlusion, resulting in lower rate of regulation values. In contrast to HFrEF,[6,7] these adjustments did not occur parallel to systolic dysfunction because resting CO was normal in the AB group. Consequently, peripheral vascular stiffening[20–23] and augmented vasoconstriction,[23,38] often observed in HFpEF and potentially related to reduced shear stress in the current study, likely contributed to the reduced resting carotid blood flow and impaired autoregulatory responses observed in AB animals. These results support our hypothesis and suggest that deficits in cognition manifest as the result of peripheral vascular dysfunction as opposed to myocardial systolic impairment. Furthermore, these findings provide additional support for the concept that HFpEF is a systemic disease affecting cardiac and noncardiac tissues alike.[24,25]

Clinical Significance

Given that arterial stiffness is characteristic of the HFpEF syndrome,[20–23] the widespread clinical accessibility of high-resolution Doppler ultrasound highlights carotid artery vascular mechanics as an easily obtainable, reproducible, and potentially valid vascular biomarker, secondary to cognitive testing itself, for the purpose of tracking, preventing, or treating (vascular) CI in a setting of HFpEF. Recent position statements from the American

Table 3. Hemodynamic Variables During Transient Vena Cava Occlusion

	Control (n=5)	Aortic-Banded Sedentary (n=6)	Aortic-Banded Exercise Trained (n=6)
Cardiac output, L·min^{-1}			
Before	7.6±1.0	6.8±0.5	7.8±0.7
5 seconds	3.3±0.3	3.8±0.2	5.2±0.4
End	2.4±0.3	2.2±0.2	3.6±0.5
Mean arterial pressure, mm Hg			
Before	60±5	80±7*	75±7*
5 seconds	41±3	60±5*	54±4*
End	25±2	31±3	28±1
Carotid blood flow, mL·min^{-1}			
Before	239±31	288±33	441±55†
5 seconds	192±31	183±26	352±42†
End	78±8	76±11	116±11
Carotid vascular resistance, mm Hg·mL·min^{-1}			
Before	0.26±0.01	0.29±0.02‡	0.18±0.02§
5 seconds	0.23±0.02	0.34±0.02‡	0.16±0.01§
End	0.34±0.04	0.44±0.06‡	0.25±0.02§

*P<0.05, group–occlusion interaction, aortic-banded sedentary and exercise trained vs control.
†P<0.05 group–occlusion interaction, aortic-banded exercise trained vs all other groups.
‡P<0.05, group main effect, aortic-banded sedentary vs all other groups.
§P<0.05, group main effect, aortic-banded exercise trained vs control.

Heart Association and the American Stroke Association stated that peripheral arterial stiffness is an emerging marker of arterial aging that may also serve as a vascular risk factor for CI.[19] Our regression analyses and ultrasound data support this conclusion because carotid artery stiffness, indicated by decreased arterial deformation, strain, and increased Peterson elastic modulus, was increased in the AB group and was significantly correlated with average memory scores. Although both HFpEF and HFrEF patients exhibit HF-induced CI, given that EF percentage and CO remain normal under resting conditions in HFpEF,[13,14] our results suggested that deficits in cognition are the result of peripheral (vascular) dysfunction (as opposed to myocardial systolic impairment) and highlighted the potential clinical value of using carotid artery stiffness as a biomarker for CI in HFpEF patients. Furthermore, our results indicated that therapy targeted at improving peripheral conduit artery stiffness and possibly downstream compliance could be beneficial for improving HF-related dementia.

Therapeutic Benefits of Chronic Exercise

Regarding the clinical efficacy of exercise in a setting of diastolic dysfunction and LV remodeling, our results showed that chronic low-intensity interval exercise training improved indices of short- and long-term memory and prevented pathological carotid artery vascular restructuring in aortic-banded miniature swine. Exercise training appears safe,[39] improves aerobic capacity and quality of life,[40–42] and increases blood flow distribution to the frontal lobe during exercise[41] in HFpEF patients. In this respect, the necessity of improving carotid artery stiffness to realize exercise-dependent improvements in health is unclear in HFpEF. In contrast to our findings, recent human studies have shown that neither pharmacological intervention[43,44] nor exercise training (walking/upper and lower limb ergometry performed at 50–70% heart rate reserve, 3 days per week for 16 weeks) improves aortic or carotid artery stiffening.[45] Furthermore, improved exercise capacity was not dependent on a reduction in carotid artery stiffness.[45] The differences between our study and human findings could be a reflection of exercise intensity. The exercise intensity used by Kitzman and colleagues[45] was relatively low compared with other studies in non-HFpEF populations that have documented exercise-induced reductions in arterial stiffness[30,46,47] and may be less intense than our interval training paradigm. These differences outline the need to study exercise at variable intensities to optimize peripheral vascular function and overall exercise capacity in HFpEF patients.

The current study is, to the best of our knowledge, the first to demonstrate exercise-dependent benefits in cognition in a large animal experimental setting consistent with HFpEF. Exercise prevented carotid artery vascular stiffening, preserved normal blood flow control, and reduced the resistive index, indicating more continuous supply of blood flow to the head during diastole, perhaps the result of increased carotid artery and downstream microvascular compliance.[27,48] Our findings reaffirmed the link between carotid artery vascular stiffening and CI[19] and highlighted the therapeutic benefit of integrating exercise training into standard care for HFpEF patients at risk of developing CI. Combining our results with those in the literature clearly suggests that both cognitive dysfunction and exercise intolerance are, to a significant degree, the result of peripheral adaptions[49–51] including those in the systemic vasculature.[25,38,49,50] Going forward, determining the mechanisms responsible for the benefits of exercise will aid in developing targeted therapeutic strategies for limiting or preventing carotid stiffening and associated CI and/or cardiogenic dementia in HF.

A Novel Large Animal Model of CI

Results from the current study lay the initial foundation for aortic banding in swine as a new large animal model of CI in a setting of diastolic dysfunction (ie, HFpEF). Swine represent a gold standard regarding translational relevance for human cardiovascular[52] and cognitive function.[53] Recent position statements from the American Heart Association and the

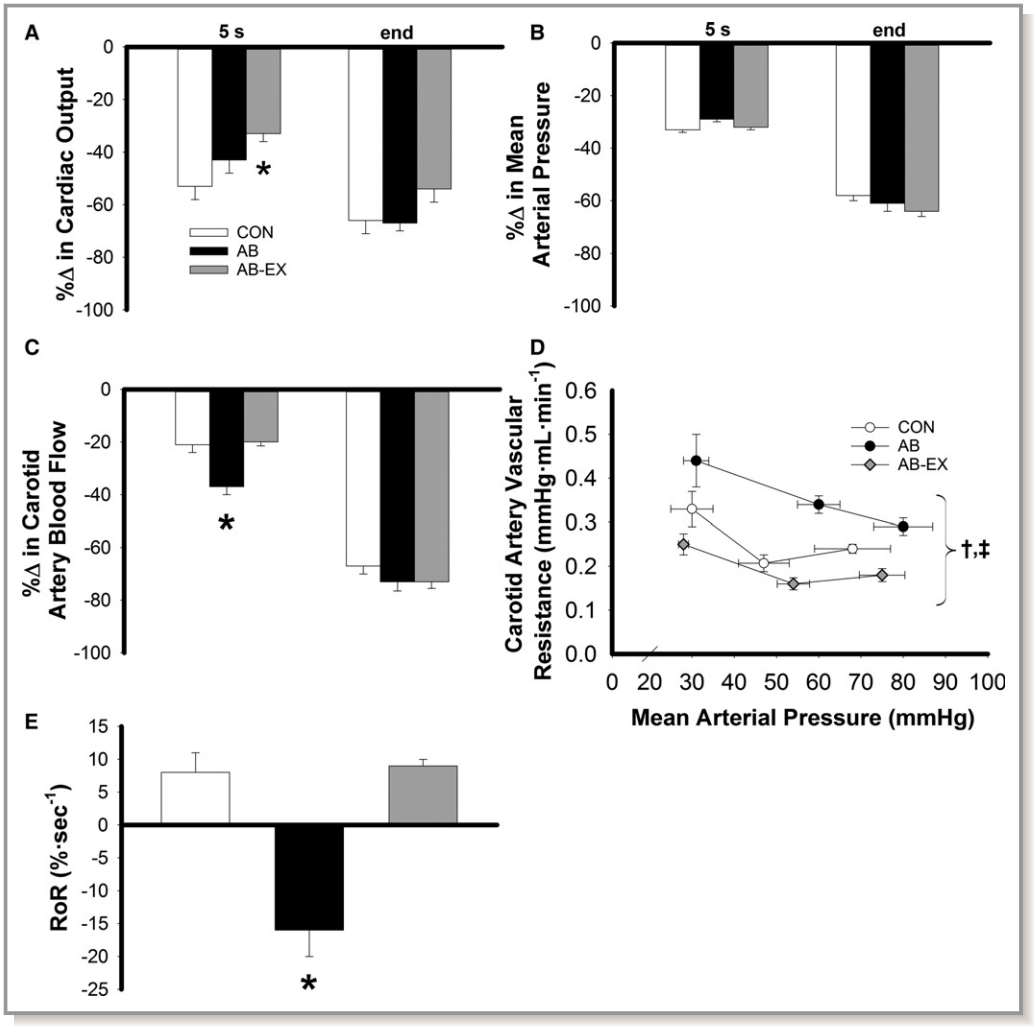

Figure 4. Percentage changes in (A) cardiac output, (B) mean arterial pressure, and (C) carotid artery blood flow; (D) changes in carotid artery vascular resistance ($^{\dagger}P<0.05$, group main effect, AB vs all other groups, $^{\ddagger}P<0.05$, group main effect, CON vs AB-EX); and (E) RoR at 5 seconds and the end of vena cava occlusion. $^{*}P<0.05$ vs all other groups. AB indicates the aortic-banded sedentary group; AB-EX, aortic-banded exercise-trained group; CON, nonsham sedentary control group; RoR, rate of regulation.

American Stroke Association indicate that models in higher order species are needed and desirable, given the limitations of rodent models in terms of small amounts of white matter and behavioral range.[19] Both pig brain and heart share similar anatomical, biochemical, and functional similarities with those of humans, including brains that are gyrencephalic, that contain >60% white matter, and that rely primarily on the carotid and middle cerebral arteries for continuous blood supply.[52–56] These features make the pig ideal for studying the relationship between peripheral/cerebral vascular function and cognition in terms of optimizing translational impact.

Considerations

Blood flow was measured in the common carotid artery, which supplies the entire head and not the cerebral circulation exclusively. The depth and steep angle of the pig internal carotid artery rendered it impossible to obtain valid measures using Doppler ultrasound at an angle of insonation <60°. To ensure the most accurate, valid, and reproducible values, we examined the common carotid artery ≈2 to 5 cm proximal to the carotid bifurcation. Common carotid artery flow overestimates cerebral flow, but flow in the 2 vascular segments exhibits a strong correlative relationship (R=0.94).[57] Given that alterations in blood flow through the common carotid artery may arise from changes in downstream vascular control in the skin, muscle, or brain, we ensured that body temperature remained stable and skeletal muscle remained inactive throughout the protocol. Advantages of examining the common carotid artery using Doppler ultrasound rest on its easy accessibility and high degree of reproducibility as well as its widespread clinical use.[21,33]

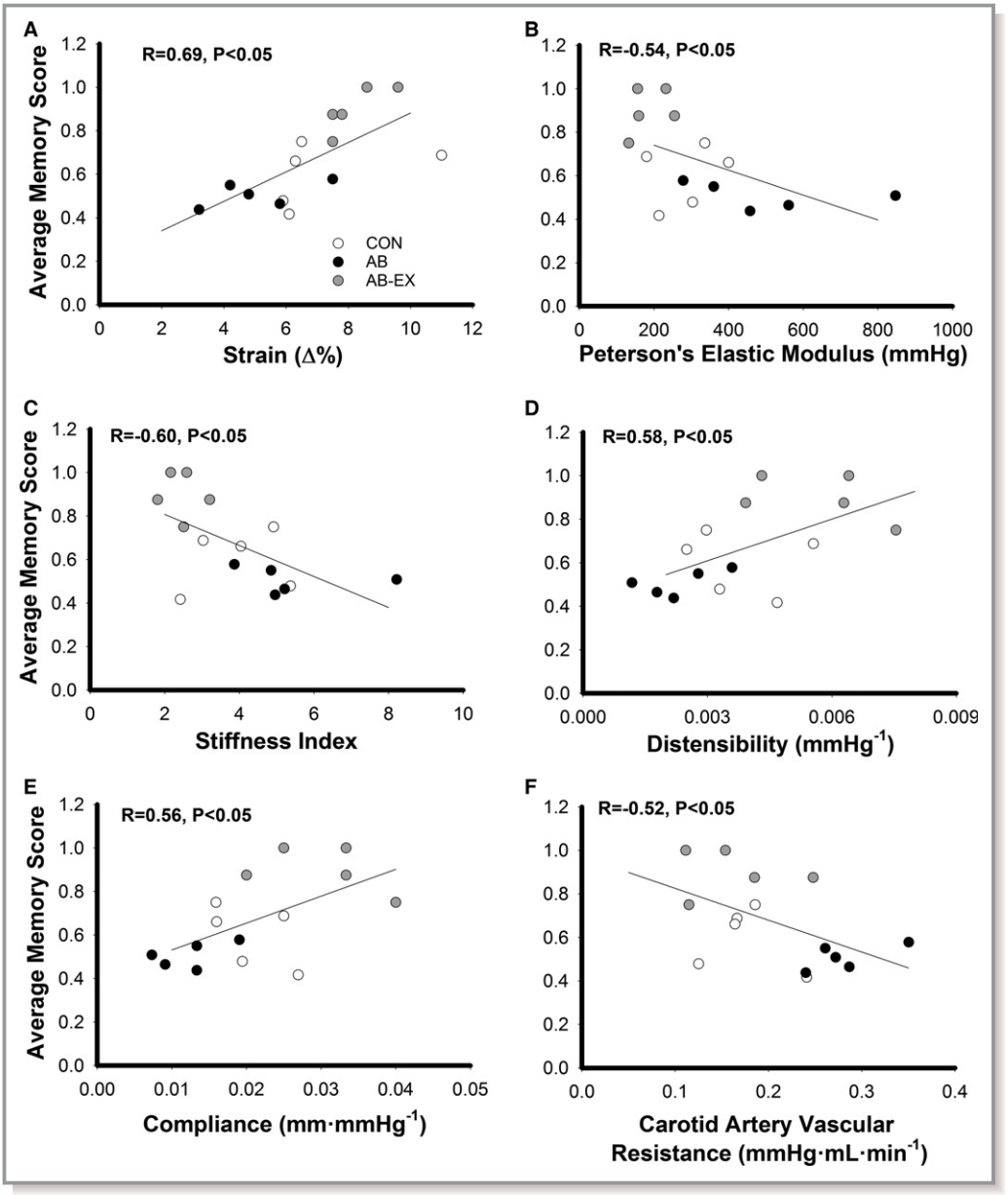

Figure 5. Linear regression analyses examining the association between carotid artery (A) strain (Δ%), (B) Peterson's elastic modulus (mmHg), (C) stiffness index, (D) distensibility ($mmHg^{-1}$) (E) compliance ($mm \cdot mmHg^{-1}$) and (F) vascular resistance ($mmHg \cdot mL \cdot min^{-1}$) vs average memory score.

Although our data support a link between peripheral vascular dysfunction and CI in a large animal model reminiscent of stable human HFpEF, they do not definitively demonstrate that CI was caused by peripheral conduit artery dysfunction alone. It is also important to note from an epidemiological perspective, compared with the current animal model, that humans with HFpEF tend to be older women that exhibit multiple comorbidities, including advanced age and type 2 diabetes.[13,14,24] Although a primary strength of our study is the use of in vivo methodology in a large animal model that provides translational impact, it limits our sample size. In total, these important perspectives underscore the importance of examining these findings in human HFpEF to validate the clinical relevance of our study.

Conclusion

We provided evidence that elevated carotid artery stiffness, cranial hypoperfusion, and impaired blood flow control are associated with deficits in cognition independent of cardiac systolic dysfunction at rest in aortic-banded miniature swine. Furthermore, chronic low-intensity interval exercise training

prevented cognitive dysfunction while preserving normal blood flow control and improving clinical indices of carotid arterial stiffness. In contrast to HFrEF, these findings suggest that HFpEF-induced dementia stems from peripheral (vascular) dysfunction and support the use of carotid artery stiffness as a biomarker and/or risk factor for cognitive dysfunction in HFpEF.

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Disclosures

None.

References

1. Abdon NJ, Malmcrona R. High pacemaker implantation rate following "cardiogenic neurology". *Acta Med Scand*. 1975;198:455–461.
2. Anonymous. Cardiogenic dementia. *Lancet*. 1977;1:27–28.
3. Shapiro W, Chawala NPS. Observations on the regulation of cerebral blood flow in complete heart block. *Circulation*. 1969;40:863–870.
4. Sensenbach W, Madison L, Eisenberg S. Cerebral hemodynamic and metabolic studies in patients with Congest Heart Fail. I. Observations in lucid subjects. *Circulation*. 1960;21:697–703.
5. Sensenbach W, Madison L, Eisenberg S. Cerebral hemodynamic and metabolic studies in patients with Congest Heart Fail. II. Observations in confused subjects. *Circulation*. 1960;21:704–709.
6. Fraser KS, Heckman GA, McKelvie RS, Harkness K, Middleton LE, Hughson RL. Cerebral hypoperfusion is exaggerated with an upright posture in heart failure. *JACC Heart Fail*. 2015;3:168–175.
7. Gruhn N, Larsen FS, Boesgaard S, Knudsen GM, Mortensen SA, Thomsen G, Aldershvile J. Cerebral blood flow in patients with chronic heart failure before and after heart transplantation. *Stroke*. 2001;32:2530–2533.
8. Pressler SJ, Subramanian U, Kareken D, Perkins SK, Gradus-Pizlo I, Sauve MJ, Ding Y, Kim J, Sloan R, Jaynes H, Shaw RM. Cognitive deficits in chronic heart failure. *Nurse Res*. 2011;59:127–139.
9. Eggermont LHP, de Boer K, Muller M, Jaschke AC, Kamp O, Scherder EJA. Cardiac disease and cognitive impairment: a systematic review. *Heart*. 2012;98:1334–1340.
10. VanDen Hurk K, Reijmer YD, Van Den Berg E, Alssema M, Nijpels G, Kostense PJ, Stehouwer CDA, Paulus WJ, Kamp O, Dekker JM, Biessels GJ. Heart failure and cognitive function in the general population: the Hoorn Study. *Eur J Heart Fail*. 2011;13:1362–1369.
11. Dodson JA, Truong TTN, Towle VR, Kerins G, Chaudhry SI. Cognitive impairment in older adults with heart failure: prevalence, documentation, and impact on outcomes. *Am J Med*. 2013;126:120–126.
12. Athilingam P, D'Aoust RF, Miller L, Chen L. Cognitive profile in persons with systolic and diastolic heart failure. *Congest Heart Fail*. 2013;19:44–50.
13. Abudiab MM, Redfield MM, Melenovsky V, Olson TP, Kass DA, Johnson BD, Borlaug BA. Cardiac output response to exercise in relation to metabolic demand in heart failure with preserved ejection fraction. *Eur J Heart Fail*. 2013;15:776–785.
14. Schwartzenberg S, Redfield MM, From AM, Sorajja P, Nishimura RA, Borlaug BA. Effects of vasodilation in heart failure with preserved or reduced ejection fraction: implications of distinct pathophysiologies on response to therapy. *J Am Coll Cardiol*. 2012;59:442–451.
15. Awad N, Gangno M, Messier C. The relationship between impaired glucose tolerance, type 2 diabetes, and cognitive function. *J Clin Exp Neuropsychol*. 2004;26:1044–1080.
16. Heckman GA, Onge JS. Heart failure and cognitive impairment: challenges and opportunities. *Clin Interv Aging*. 2007;2:209–218.
17. Borlaug BA, Paulus WJ. Heart failure with preserved ejection fraction: pathophysiology, diagnosis, and treatment. *Eur Heart J*. 2011;32:670–679.
18. Murad K, Goff DC, Morgan TM, Burke GL, Bartz TM, Kizer JR, Chaudhry SI, Gottdiener JS, Kitzman DW. Burden of comorbidities and functional and cognitive impairments in elderly patients at the initial diagnosis of heart failure and their impact on total mortality: the Cardiovascular Health Study. *JACC Heart Fail*. 2015;3:542–550.
19. Gorelick PB, Scuteri A, Black SE, DeCarli C, Greenberg SM, Iadecola C, Launer LJ, Laurent S, Lopez OL, Nyenhuis D, Peterson RC, Schneider JA, Tzourio C, Arnett DK, Bennett DA, Chui HC, Higashida RT, Lindquist R, Nilsson PM, Roman GC, Sellke FW, Seshadri S. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2011;42:2672–2713.
20. Hundley WG, Kitzman DW, Morgan TM, Hamilton CA, Darty SN, Stewart KP, Herrington DM, Link KM, Little WC. Cardiac cycle-dependent changes in aortic area and distensibility are reduced in older patients with isolated diastolic heart failure and correlate with exercise intolerance. *J Am Coll Cardiol*. 2001;38:796–802.
21. Kitzman DW, Herrington DM, Brubaker PH, Moore JB, Eggebeen J, Haykowsky MJ. Carotid arterial stiffness and its relationship to exercise intolerance in older patients with heart failure and preserved ejection fraction. *Hypertension*. 2013;61:112–119.
22. Tartière-Kesri L, Tartière J-M, Logeart D, Beauvais F, Cohen Solal A. Increased proximal arterial stiffness and cardiac response with moderate exercise in patients with heart failure and preserved ejection fraction. *J Am Coll Cardiol*. 2012;59:455–461.
23. Weber T, Wassertheurer S, O'Rourke MF, Haiden A, Zweiker R, Rammer M, Hametner B, Eber B. Pulsatile hemodynamics in patients with exertional dyspnea: potentially of value in the diagnostic evaluation of suspected heart failure with preserved ejection fraction. *J Am Coll Cardiol*. 2013;61:1874–1883.
24. Paulus WJ, Tschöpe C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. *J Am Coll Cardiol*. 2013;62:263–271.
25. Upadhya B, Haykowsky MJ, Eggebeen J, Kitzman DW. Exercise intolerance in heart failure with preserved ejection fraction: more than a heart problem. *J Geriatr Cardiol*. 2015;12:294–304.
26. Tarumi T, Gonzales MM, Fallow B, Nualnim N, Pyron M, Tanaka H, Haley AP. Central artery stiffness, neuropsychological function, and cerebral perfusion in sedentary and endurance-trained middle-aged adults. *J Hypertens*. 2013;31:2400–2409.
27. van Sloten TT, Protogerou AD, Henry RMA, Schram MT, Launer LJ, Stehouwer CDA. Association between arterial stiffness, cerebral small vessel disease and cognitive impairment: a systematic review and meta-analysis. *Neurosci Biobehav Rev*. 2015;53:121–130.
28. Marshall KD, Muller BN, Krenz M, Hanft LM, McDonald KS, Dellsperger KC, Emter CA. Heart failure with preserved ejection fraction: chronic low-intensity interval exercise training preserves myocardial O2 balance and diastolic function. *J Appl Physiol*. 2013;114:131–147.
29. Emter CA, Baines CP. Low-intensity aerobic interval training attenuates pathological left ventricular remodeling and mitochondrial dysfunction in aortic-banded miniature swine. *Am J Physiol Heart Circ Physiol*. 2010;299:H1348–H1356.
30. Tanaka H, Dinenno FA, Monahan KD, Clevenger CM, DeSouza CA, Seals DR. Aging, habitual exercise, and dynamic arterial compliance. *Circulation*. 2000;102:1270–1275.
31. Erickson KI, Voss MW, Prakash RS, Basak C, Szabo A, Chaddock L, Kim JS, Heo S, Alves H, White SM, Wojcicki TR, Mailey E, Vieira VJ, Martin SA, Pence BD, Woods JA, McAuley E, Kramer AF. Exercise training increases size of hippocampus and improves memory. *Proc Natl Acad Sci USA*. 2011;108:3017–3022.
32. Lagerkranser M, Stange K, Sollevi A. Effects of propofol on cerebral blood flow, metabolism and cerebral auoregulation in the anesthetized pig. *J Neurosurg Anesthesiol*. 1997;9:188–193.
33. O'Rourke MF, Staessen JA, Vlachopoulos C, Duprez D, Plante GE. Clinical applications of arterial stiffness; definitions and reference values. *Am J Hypertens*. 2002;15:426–444.
34. Darby R, Chhabra RP. *Chemical Engineering Fluid Mechanics*. 2nd ed. New York, NY: CRC Press; 2001.

35. Aaslid R, Lindegaard KF, Sorteberg W, Nornes H. Cerebral autoregulation dynamics in humans. *Stroke*. 1989;20:45–52.
36. Bolhuis JE, Oostindjer M, Hoeks CWF, de Haas EN, Bartels AC, Ooms M, Kemp B. Working and reference memory of pigs (Sus scrofa domesticus) in a holeboard spatial discrimination task: the influence of environmental enrichment. *Anim Cogn*. 2013;16:845–850.
37. van der Staay FJ, Gieling ET, Pinzón NE, Nordquist RE, Ohl F. The appetitively motivated "cognitive" holeboard: a family of complex spatial discrimination tasks for assessing learning and memory. *Neurosci Biobehav Rev*. 2012;36:379–403.
38. Borlaug BA, Melenovsky V, Russell SD, Kessler K, Pacak K, Becker LC, Kass DA. Impaired chronotropic and vasodilator reserves limit exercise capacity in patients with heart failure and a preserved ejection fraction. *Circulation*. 2006;114:2138–2147.
39. Kitzman DW, Brubaker P, Morgan T, Haykowsky M, Hundley G, Kraus WE, Eggebeen J, Nicklas BJ. Effect of caloric restriction or aerobic exercise training on peak oxygen consumption and quality of life in obese older patients with heart failure with preserved ejection fraction: a randomized clinical trial. *JAMA*. 2016;315:36–46.
40. Kitzman DW, Brubaker PH, Morgan TM, Stewart KP, Little WC. Exercise training in older patients with heart failure and preserved ejection fraction: a randomized, controlled, single-blind trial. *Circ Heart Fail*. 2011;3:659–667.
41. Fu T-C, Yang N-I, Wang C-H, Cherng W-J, Chou S-L, Pan T-L, Wang J-S. Aerobic interval training elicits different hemodynamic adaptations between heart failure patients with preserved and reduced ejection fraction. *Am J Phys Med Rehabil*. 2016;95:15–27.
42. Edelmann F, Gelbrich G, Düngen H-D, Fröhling S, Wachter R, Stahrenberg R, Binder L, Töpper A, Lashki DJ, Schwarz S, Herrmann-Lingen C, Löffler M, Hasenfuss G, Halle M, Pieske B. Exercise training improves exercise capacity and diastolic function in patients with heart failure with preserved ejection fraction: results of the Ex-DHF (Exercise training in Diastolic Heart Failure) pilot study. *J Am Coll Cardiol*. 2011;58:1780–1791.
43. Kitzman DW, Hundley WG, Brubaker PH, Timothy M, Moore JB, Stewart KP, Little WC. A randomized double-blind trial of enalapril in older patients with heart failure and preseved ejection fraction: effects on exercise tolerance and arterial distensibility. *Circ Heart Fail*. 2010;3:477–485.
44. Little WC, Zile MR, Kitzman DW, Hundley WG, O'Brien TX, Degroof RC. The effect of alagebrium chloride (ALT-711), a novel glucose cross-link breaker, in the treatment of elderly patients with diastolic heart failure. *J Card Fail*. 2005;11:191–195.
45. Kitzman DW, Brubaker PH, Herrington DM, Timothy M, Stewart KP, Hundley WG, Haykowsky MJ. Effect of endurance exercise training on endothelial function and arterial stiffness in older patients with heart failure and preserved ejection fraction: a randomized, controlled, single-blind trial. *J Am Coll Cardiol*. 2014;62:584–592.
46. Moreau KL, Donato AJ, Seals DR, DeSouza CA, Tanaka H. Regular exercise, hormone replacement therapy and the age-related decline in carotid arterial compliance in healthy women. *Cardiovasc Res*. 2003;57:861–868.
47. Woolley B, Stoner L, Lark S, Wong L, Lanford J, Faulkner J. Effect of early exercise engagement on arterial stiffness in patients diagnosed with a transient ischaemic attack. *J Hum Hypertens*. 2015;29:87–91.
48. Bude RO, Rubin JM. Relationship between the resistive index and vascular compliance and resistance. *Radiology*. 1999;211:411–417.
49. Haykowsky MJ, Brubaker PH, John JM, Stewart KP, Morgan TM, Kitzman DW. Determinants of exercise intolerance in elderly heart failure patients with preserved ejection fraction. *J Am Coll Cardiol*. 2011;58:265–274.
50. Haykowsky MJ, Kouba EJ, Brubaker PH, Nicklas BJ, Eggebeen J, Kitzman DW. Skeletal muscle composition and its relation to exercise intolerance in older patients with heart failure and preserved ejection fraction. *Am J Cardiol*. 2014;113:1211–1216.
51. Bowen TS, Rolim NPL, Fischer T, Baekkerud FH, Medeiros A, Werner S, Brønstad E, Rognmo O, Mangner N, Linke A, Schuler G, Silva GJJ, Wisloff U, Adams V. Heart failure with preserved ejection fraction induces molecular, mitochondrial, histological, and functional alterations in rat respiratory and limb skeletal muscle. *Eur J Heart Fail*. 2015;17:263–272.
52. Douglas WR. Of pigs and men and research: a review of applications and analogies of the pig, sus scrofa, in human medical research. *Space Life Sci*. 1972;3:226–234.
53. Kornum BR, Knudsen GM. Cognitive testing of pigs (Sus scrofa) in translational biobehavioral research. *Neurosci Biobehav Rev*. 2011;35:437–451.
54. Lind NM, Moustgaard A, Jelsing J, Vajta G, Cumming P, Hansen AK. The use of pigs in neuroscience: modeling brain disorders. *Neurosci Biobehav Rev*. 2007;31:728–751.
55. Swindle MM, Makin A, Herron AJ, Clubb FJ, Frazier KS. Swine as models in biomedical research and toxicology testing. *Vet Pathol*. 2012;49:344–356.
56. Platt SR, Holmes SP, Howerth EW, Duberstein KJJ, Dove CR, Kinder HA, Wyatt EL, Linville AV, Lau VW, Stice SL, Hill WD, Hess DC, West FD. Development and characterization of a Yucatan miniature biomedical pig permanent middle cerebral artery occlusion stroke model. *Exp Transl Stroke Med*. 2014;6:5.
57. Meadow W, Rudinsky B, Raju T, John E, Fornell L, Shankararao R. Correlation of flow probe determinations of common carotid artery blood flow and internal carotid artery blood flow with microsphere determinations of cerebral blood flow in piglets. *Pediatr Res*. 1999;45:324–330.