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RESEARCH ARTICLE

Glycine improves peritoneal vasoreactivity to dialysis solutions in the elderly

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ABSTRACT

Background: Peritoneal dialysis solution (PDS) dilates peritoneal microvessels predominantly by the activation of the endothelial nitric oxide (NO) pathway. We made an incidental observation of decreased PDS-induced, NO-dependent peritoneal microvascular vasoreactivity in elderly rats naïve to PDS exposure. We hypothesized that this subordinate NO-mediated peritoneal microvascular vasoreactivity is caused by increased oxidative stress in the aged endothelium, which compromises NO bioavailability in the elderly, and that peritoneal microvascular vasoreactivity can be improved by the supplementation of antioxidant glycine to PDS.
Methods: We studied PDS-mediated vasoreactivity of four intestinal visceral arterioles of different orders by *in vivo* intravital microscopy in weaned, adult, and elderly rats to (i) confirm subordinate vasoreactivity to PDS in elderly rats; (ii) restore vasoreactivity by glycine supplementation; and (iii) establish age as an independent risk factor for endothelial cell dysfunction.
Results: In a crossover series, peritoneal microvascular vasoreactivity to PDS exposure was remarkably decreased in elderly rats. This subordinate vasoreactivity was completely restored by the supplementation of glycine to PDS. In a separate series, we assessed *in situ* endothelial cell function in weaned and adult rats using the cumulative acetylcholine concentration – response curves. Unlike the adults, the weaned rats demonstrated remarkable sensitivity and reactivity to cumulative acetylcholine concentrations, suggesting the dependency of endothelial cell function on age.
Conclusion: Aging is an independent risk factor for peritoneal microvascular endothelial cell dysfunction. Endothelial function in the elderly can be recovered by

reinforcing the bioavailability of endothelial-derived NO through glycine. Dietary glycine supplementation is a potential therapeutic strategy to decrease the burden of oxidative stress on the aged endothelium.

Keywords: intravital microscopy, peritoneal microcirculation, endothelial cell, aging, glycine

INTRODUCTION

Peritoneal dialysis (PD) is a form of renal replacement therapy for patients with end-stage renal failure. This continuous maintenance therapy requires repeated intraperitoneal instillation and drainage of a clinical glucose-based peritoneal dialysis solution (PDS). When dwelling in the peritoneal cavity, PDS removes excess body water by osmosis and uremic toxins by diffusion through equilibration exchange across the blood – peritoneal barrier (peritoneal membrane). Acute exposure of the peritoneum to PDS produces rapid and sustained peritoneal microvascular vasodilation. Hyperosmolality, glucose content, lactate at low pH, and glucose degradation products collectively produce the vasodilatory action of PDS.[1,2] Moreover, peritoneal microvascular vasodilation is predominantly mediated by endothelium-dependent mechanisms involving primary and secondary nitric oxide (NO) release for vasodilation.[3] As reviewed elsewhere, the microvascular endothelium releases components causing dilation as well as constriction to control the local microvascular tone.[4] The dilation components of the microvascular tone are NO, prostacyclin, and endothelium-derived hyperpolarizing factor; the constriction components are thromboxane and endothelin-1. Recently, we made an incidental observation that the peritoneal microvascular response to PDS is impaired in elderly rats naïve to PDS exposure. Since PDS-induced vasodilation is entirely accounted for by NO, this impairment of PDS-induced dilation must be explained by a limitation in the endothelial NO pathway in the elderly. Provided that impaired endothelial-dependent dilation can be quantitatively measured, any impaired dilation response qualifies as an index of endothelial cell dysfunction. In fact, this is the rationale for the measurement of endothelial cell function *in vivo*. Measurements of dilation in response to an exogenous stimulus, including an endothelial-dependent agonist or a vasoactive solution such as PDS, are typically used to assess endothelial cell function.

It is recognized that vascular aging is an independent risk factor for age-related cardiovascular events, such as atherothrombosis, stroke, myocardial infarction, ischemic colitis, and heart failure.[5,6] The hallmark of vascular aging is endothelial cell senescence.[7] Studies on the mechanisms of aging-related endothelial cell dysfunction have been predominantly performed in *ex vivo* and *in vitro* models to identify potential targets for therapeutic intervention.[7–14] Although many therapeutic agents targeting vascular inflammation, angiogenesis, and oxidative stress have emerged from these studies, their translation to clinical use is still under development. This is attributed to the rather complex pathophysiology of vascular aging, which involves cellular, subcellular, and molecular mechanisms that interact with other compounding risk factors prevalent in old age, such as hypercholesterolemia, hypertension, and diabetes.[15] To fully account for the microvascular functions prevailing in aging, an *in vivo* model comparing these functions in adult and elderly animals is required. The gold standard for the real-time *in vivo* study of microvascular and endothelial cell functions is intravital microscopy (IVM). This technique allows for real-time quantitative measurement of microvascular reactivity to exogenous stimuli such as PDS as well as for the simultaneous assessment of the prevailing endothelial and vascular smooth muscle functions that produce the observed reactivity. This study utilized direct IVM to examine the aging-dependent decline in endothelial cell function and to test the hypotheses that (1) the vasoreactivity of the visceral peritoneal microvasculature to PDS is reduced in elderly rats due to the increased burden of oxidative stress that compromises endothelial NO bioavailability and (2) the subordinate vasoreactivity can be prevented by antioxidant glycine.

MATERIALS AND METHODS

Animal care and surgical preparation

The Institutional Animal Care and Use Committees of Hamad Medical Corporation and Qatar University (Doha, Qatar) preapproved the experimental protocols. All animals received humane care in accordance with The Guide for the Care and Use of Laboratory Animals.[16] Male Sprague Dawley rats aged three weeks (weaned), eight weeks (adults), and two years (elderly) were procured from Harlan Laboratories (Correzzana, Italy). The animals were

quarantined for one week in a temperature- and light-controlled environment, during which they were provided with standard rat chow food and water *ad libitum*. On the experiment day, the rats were anesthetized with an intraperitoneal injection of 50 mg/kg pentobarbitone (Troy Laboratories Pvt. Ltd., Plumpton, Australia). Anesthesia was maintained during the experiments with supplemental subcutaneous injections of 0.06 mL as 25% of the induction dose at 60-minute intervals. Body temperature was maintained at 37° ± 0.5°C with a rectal probe and a servo-controlled heating pad (TC-1000, Temperature Controller, CWE Incorporated, Ardmore, PA, USA). The operation was performed after the complete loss of blink and withdrawal reflexes. Upon performing tracheostomy, a polyethylene PE-240 tubing (Scientific Commodities Inc., Lake Havasu City, AZ, USA) was introduced into the trachea to ensure the patency of the upper airway and that the rats inhaled room air spontaneously. The left carotid artery was cannulated with PE-50 tubing (Instech Laboratories, Inc., Plymouth Meeting, PA, USA) for continuous blood pressure monitoring using a calibrated pressure transducer connected to a blood pressure analyzer (BPA-400, Digi-Med, Louisville, KY, USA).

Solutions and drugs

Glucose-free, nonvasoactive Krebs solution containing 6.92 g/L sodium chloride, 0.44 g/L potassium chloride, 0.37 g/L calcium chloride, and 2.1 g/L sodium bicarbonate at a pH of 7.4 and an osmolarity of 285 mOsm/L was used continuously to bathe the intestinal segment during tissue preparation, during equilibration periods, and for washouts. A dextrose-based PDS (2.27%) (Dianeal PD4: Baxter, Damam, Saudi Arabia) was used containing 5.38 g/L sodium chloride, 4.48 g/L sodium lactate, 0.184 g/L calcium chloride, and 0.051 g/L magnesium chloride at a pH of 5 – 6 and an osmolality of 395 mOsm/L. Acetylcholine hydrobromide and sodium nitroprusside were purchased from Sigma-Aldrich (St. Louis, MO, USA).

IVM

The experiments were conducted using direct *in vivo* IVM of four arterioles of different orders in the terminal ileum of the rats (Figure 1) and as explained in our previous studies.[1,3] In brief, a short segment of the terminal ileum close to the ileum – cecum junction was consistently selected and transilluminated via an optical port while continuously submerged in a Krebs solution or PDS. Bath pH and

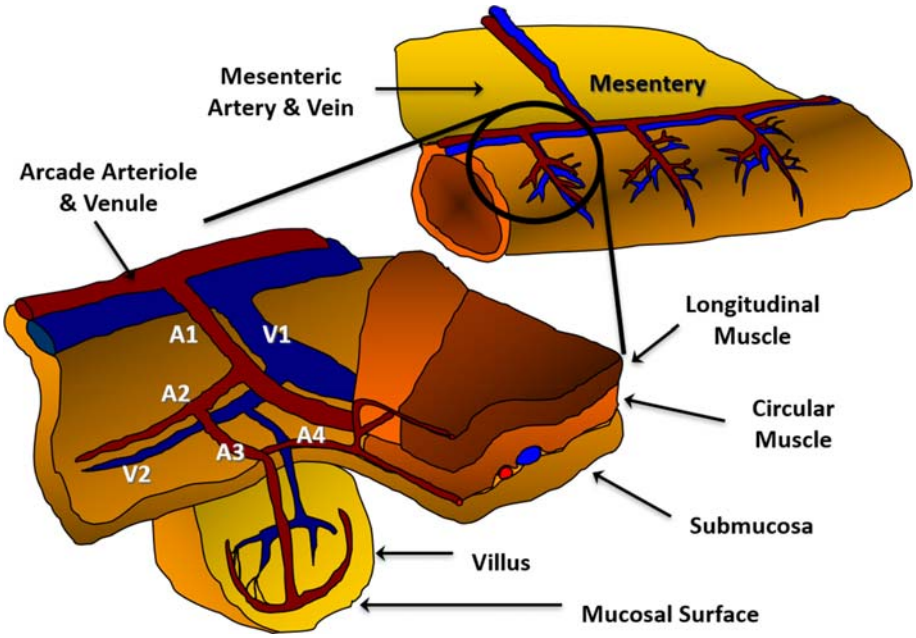

Figure 1. Anatomical organization and nomenclature of the microcirculation of the terminal ileum in rodents. A1 = first-order inflow arteriole (~100 μm); A2 = second-order arteriole (~40 – 60 μm); A3 = third-order premucosal arteriole (~15 – 20 μm); A4 = fourth-order premucosal precapillary arteriole (~7 – 10 μm). The venous microvascular tree parallels the arterial tree.

temperature were controlled and maintained by continuous bubbling of the tissue bath solution with a mixture of N_2 and CO_2 and by a temperature coil feedback system, respectively. Four arterioles of different orders [First (A1) through fourth (A4)] (100 – 8-μm diameter), as characterized by Bolen and Gore,[17] were continuously monitored in real time with an upright microscope (Axio Varioscope, Zeiss, Germany) fitted with a high-definition digital camera (Model HV-D30, Hitachi Kokusai, Tokyo, Japan). Images of the arterioles were recorded in a streamline video that was automatically saved to the computer hard drive for offline measurements of diameter by digital calipers.

Experimental protocols

Series 1

The objective of this crossover series was twofold: (1) to confirm the subordinate vasoreactivity of the aged microvasculature upon exposure to PDS and (2) to demonstrate that exposure of the aged microvasculature to PDS supplemented with glycine (antioxidant agent) restores the vasoreactivity of the microvasculature. These experiments were conducted using IVM in two-year-old rats according to the timeline protocol illustrated in Figure 2A. Following baseline arteriolar measurements, 2.27% glucose-based PDS was added to the tissue bath and the diameters of A1, A2, A3, and A4 were determined at 12- to 15-minute intervals over a period of 60 minutes. The PDS was drained, and the intestinal segment was washed three times with warm Krebs solution. Following this, PDS supplemented with glycine (final concentration, 0.17 g/60 mL) was instilled in the tissue bath, and the diameters of A1, A2, A3, and A4 were determined at 12- to 15-minute intervals over a period of 60 minutes. Finally, the endothelial cell and vascular smooth muscle functions of A1, A2, A3, and A4 were assessed based on cumulative acetylcholine concentrations (10^{-8} to 10^{-3} M) sequentially administered in the tissue bath at 10-minute intervals between doses. After the last acetylcholine dose, a single dose of sodium nitroprusside (10^{-4} M) was administered in the tissue bath to assess vascular smooth muscle function and to determine the maximum dilation capacity of each microvessel.

Series 2

The objective of this control series was twofold: to determine whether (1) glycine alone exerts any vasoactive effects on the aged microvasculature and

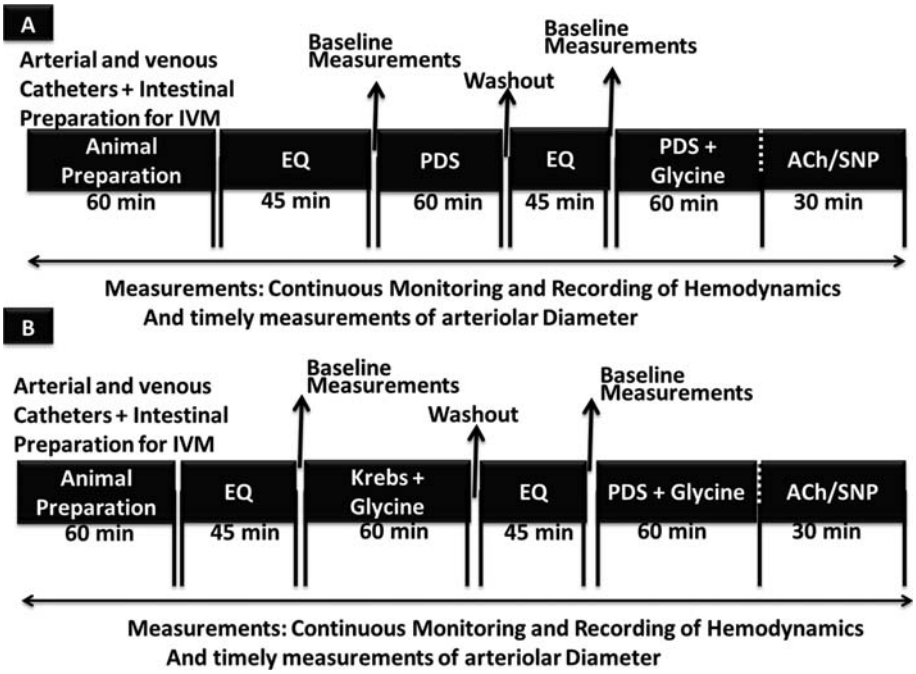

Figure 2. Timeline procedures and experimental protocols for series 1 (A) and series 2 (B). EQ = equilibration period; PDS = peritoneal dialysis solution; ACh = acetylcholine (10^{-8} to 10^{-3} M); SNP = sodium nitroprusside (10^{-4} M). ACh is the endothelium-dependent, receptor-dependent agonist (endothelial cell function); SNP is the endothelium-independent, receptor-independent agonist (vascular smooth muscle function).

(2) exposure of the aged microvasculature to PDS supplemented with glycine triggers a PDS-mediated dilation independent of glycine. This series was conducted according to the experimental protocol illustrated in Figure 2B. The animal attributes, experimental procedures, measurements, and timeline were similar to those in series 1. However, in this series, both the control and PDS were supplemented with the same glycine dose in the tissue bath (0.17 g/60 mL).

Series 3

The objective of this control series was to demonstrate a decline in endothelial cell function with age. The endothelial and vascular smooth muscle functions of A1, A2, A3, and A4 in the terminal ileum of weaned (three-week old) and adult (eight-week old) rats were studied using IVM after 60-minute exposure to a nonvasoactive Krebs solution in the tissue bath. Endothelial cell function was assessed from the cumulative concentrations of the endothelial-dependent, receptor-dependent agonist acetylcholine (10^{-8} to 10^{-4} M) sequentially administered in the tissue bath at 10-minute intervals between doses. After the last acetylcholine dose, a single dose of the endothelial-independent, receptor-independent agonist sodium nitroprusside (10^{-4} M) was administered in the tissue bath to assess vascular smooth muscle function and to determine the maximum dilation capacity of each microvessel.

Data analysis and statistics

All data are presented as mean and standard error of the mean unless stated otherwise. Microvascular diameter data were normalized and presented as percentage changes from baseline. Percentage changes in vessel diameter from baseline were assessed by one-way analysis of variance, and Dunnett multiple-range test was used to evaluate changes from baseline in the same animal. Data on acetylcholine concentration versus arteriolar response were normalized as percentage changes from maximum dilation capacity for each microvessel. The logarithm of the concentration versus arteriolar response data was fitted to a symmetrical sigmoidal function [Y = Bottom + (Top − Bottom)/(1 + 10^((LogEC50 − X)))] to determine the effective agonist concentration that provoked a response halfway between the basal (bottom) and the maximal (top) responses using GraphPad Prism version 6.00 for Windows (GraphPad Software, San Diego, CA, USA). This model assumes that the concentration – response curve has a standard slope, equal to a Hill slope (or slope factor) of 1.0. A result was considered significant if the probability of a type 1 error was $<5\%$ ($p < 0.05$).

RESULTS

In vivo effects of glycine on microvascular vasoreactivity in elderly rats

Panels A and B of Figures 3 – 6 demonstrate the intestinal microvascular vasoreactivity of A1, A2, A3, and A4 to PDS without and with glycine supplementation. The bottom panels of the figures show the vasoreactivity to control Krebs solution supplemented with glycine and PDS supplemented with glycine. The control solution with or without glycine supplementation exerted no effect on the vasoreactivity of the arterioles of any order as seen in the four figures. Additionally, in elderly rats, the initial and maximum changes in arteriolar diameter from baseline during a 60-minute exposure to PDS were only 10% and 35%, respectively. Supplementation of glycine to PDS increased the initial response by 30% from baseline and the maximum response by 50% from baseline, suggesting increased microvascular sensitivity and vasoreactivity of the arterioles to PDS after glycine supplementation. Characteristically, the arteriolar response to PDS was rapid initially and was more remarkable in the smaller premucosal arterioles than in the larger upstream arterioles. Supplementation of PDS with glycine increased the sensitivity and vasoreactivity of the arterioles to PDS and caused near maximal dilation at 60 minutes of all arteriolar orders.

Effects of glycine on the aged endothelium

Figure 7 shows the optimum nonlinear curve fitted to the experimental log concentration of acetylcholine (10^{-8} to 10^{-3} M), normalized in the y-axis as percentage change from the maximum dilation capacity, for A1, A2, A3, and A4. The parameters that determine the shape of the acetylcholine concentration – response curve are summarized in the table included in Figure 7 legend. There were no significant differences in the sensitivity (bottom) and reactivity (top) of the four arterioles to acetylcholine. Similarly, the effective acetylcholine concentration that produced a 50% response in the bottom and top

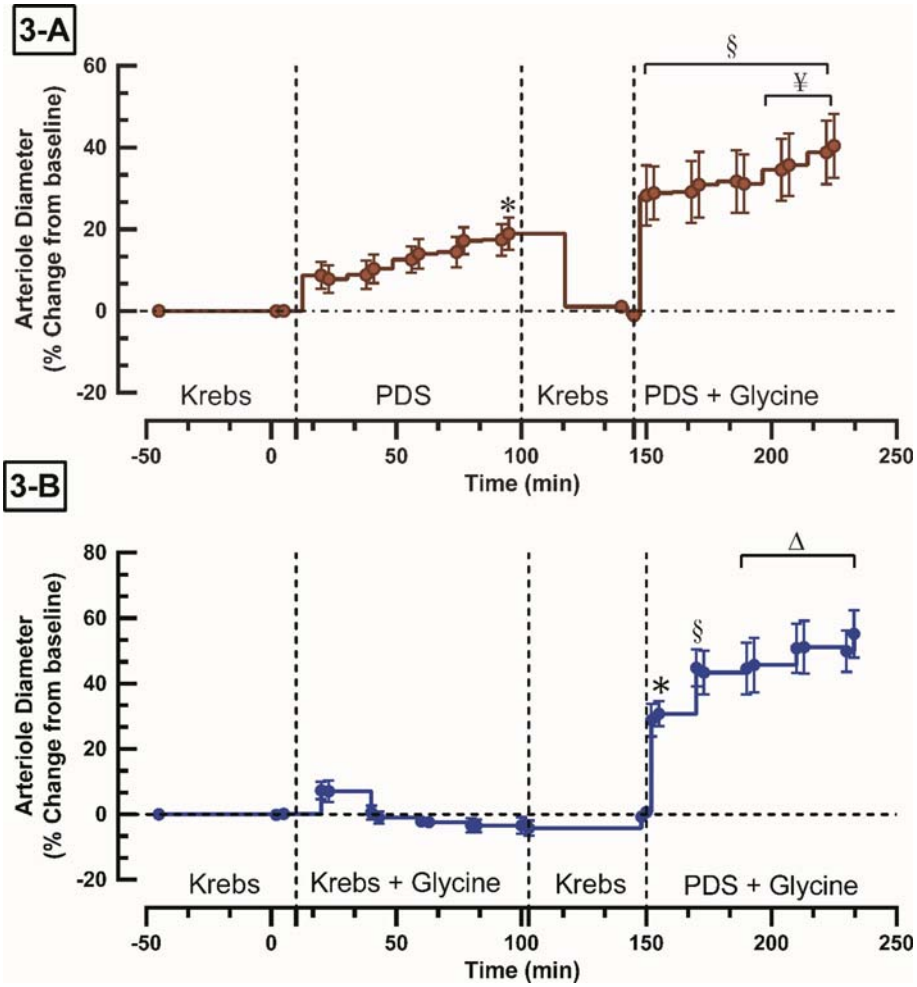

Figure 3. First-order (A1) arteriolar reactivity. Series 1 (3A): Intestinal inflow A1 arteriole (~100-μm diameter) reactivity to peritoneal dialysis solution (PDS) and PDS supplemented with glycine (PDS + Glycine). All diameter data were normalized and presented as percentage changes from baseline. Series 2 (3B): Inflow A1 arteriole response to Krebs solution supplemented with glycine (Krebs + Glycine) and PDS supplemented with glycine (PDS + Glycine). PDS is a clinical peritoneal dialysis solution (Dianeal 2.27% dextrose solution). Krebs solution is a nonvasoactive salt-balanced solution without glucose. $*p < 0.01$ versus baseline; $^{§}p < 0.001$ and $^{Δ}p < 0.0001$ versus Krebs + Glycine; $^{§}p < 0.001$ and $^{¥}p < 0.0001$ versus PDS, by two-way analysis of variance and post Dunett test with correction for multiple comparisons.

of the curves was similar across the four arterioles. Assessment of the concentration–response curves shown in Figure 7 was conducted after 60 minutes of continuous exposure of the microvasculature to PDS supplemented with glycine in the tissue bath, which explains the lower span of the curves.

Age-dependent endothelial cell dysfunction

Figures 8 and 9 present the *in vivo* determination of the endothelial and vascular smooth muscle cell functions of the four arterioles in naïve weaned and adult rats. The figures show the optimum nonlinear curve fit obtained by the least-squares regression method of the experimental cumulative acetylcholine log concentration plotted versus the arteriolar response (y-axis). The A1 inflow, A2, premucosal A3, and A4 premucosal precapillary arterioles (Figures 8 and 9) in weaned and adult rats are depicted. The insets of Figures 8 and 9 represent the effective acetylcholine concentration that produced a 50% arteriolar response in the bottom and top of each agonist concentration–response curve, respectively. Arterioles in weaned rats showed significantly higher sensitivity and reactivity to acetylcholine than did the corresponding arterioles in adult rats (Figures 8 and 9). In the latter, there was a complete shift to the right of the concentration–response curves for all arterioles, suggesting decreased arteriolar

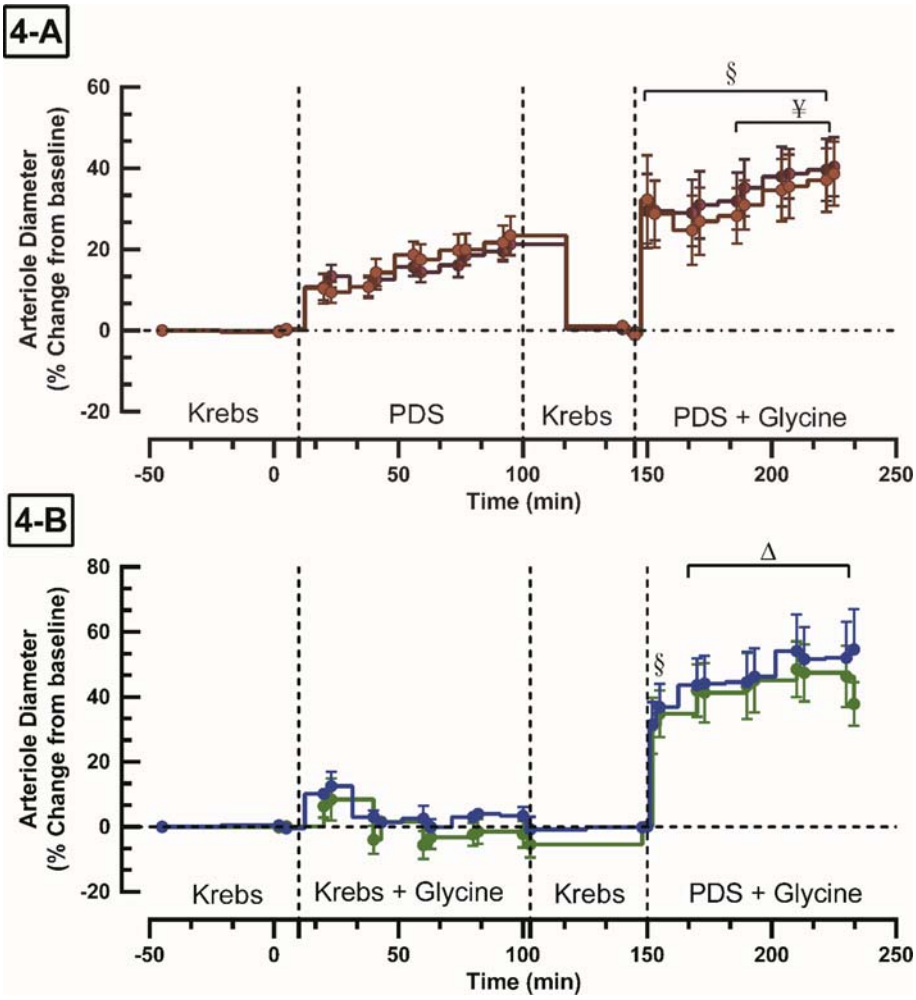

Figure 4. Second-order (A2) arteriolar reactivity. Series 1 (4A): Intestinal right and left A2 branch arterioles (~40–60-μm diameter) response to peritoneal dialysis solution (PDS) and PDS supplemented with glycine (PDS + Glycine). Series 2 (4B): Intestinal A2 arterioles response to Krebs solution supplemented with glycine (Krebs + Glycine) and PDS supplemented with glycine (PDS + Glycine). All diameter data were normalized and presented as percentage changes from baseline. PDS is a clinical peritoneal dialysis solution (Dianeal 2.27% dextrose solution). Krebs solution is a nonvasoactive salt-balanced solution containing no glucose. $*p < 0.01$ versus baseline; $^{§}p < 0.001$ and $^{Δ}p < 0.0001$ versus Krebs + Glycine; $^{§}p < 0.001$ and $^{¥}p < 0.0001$ versus PDS, by two-way analysis of variance and post Dunett test with correction for multiple comparisons.

vasoreactivity to acetylcholine. Moreover, the microvascular endothelium in the adult rats required a greater order of magnitude of acetylcholine concentration to produce a 50% arteriolar response (Insets in Figures 8A and B; Figures 9A and B).

DISCUSSION

The blood–peritoneal barrier, termed the peritoneal membrane for simplicity, constitutes a parallel series of structures that separate the blood circulating in the capillaries from free PDS in the peritoneal cavity. During PD, water and solutes are transported by osmosis-driven filtration and diffusion/convection, respectively, through three sets of pores located in the capillary wall and then across the interstitium and mesothelial cell layers to free PDS in the peritoneal cavity. Based on this membrane model, the transport rate is largely determined by the permeability of the capillary wall and the number of perfused capillaries, which determine the capillary surface area available for exchange.[18,19] Vasodilation of the precapillary arterioles promotes capillary filling and remarkably recruits capillaries, thereby increasing the effective capillary surface area available for exchange and directly impacting the peritoneal transport rate. However, for this PDS-mediated vasodilation to

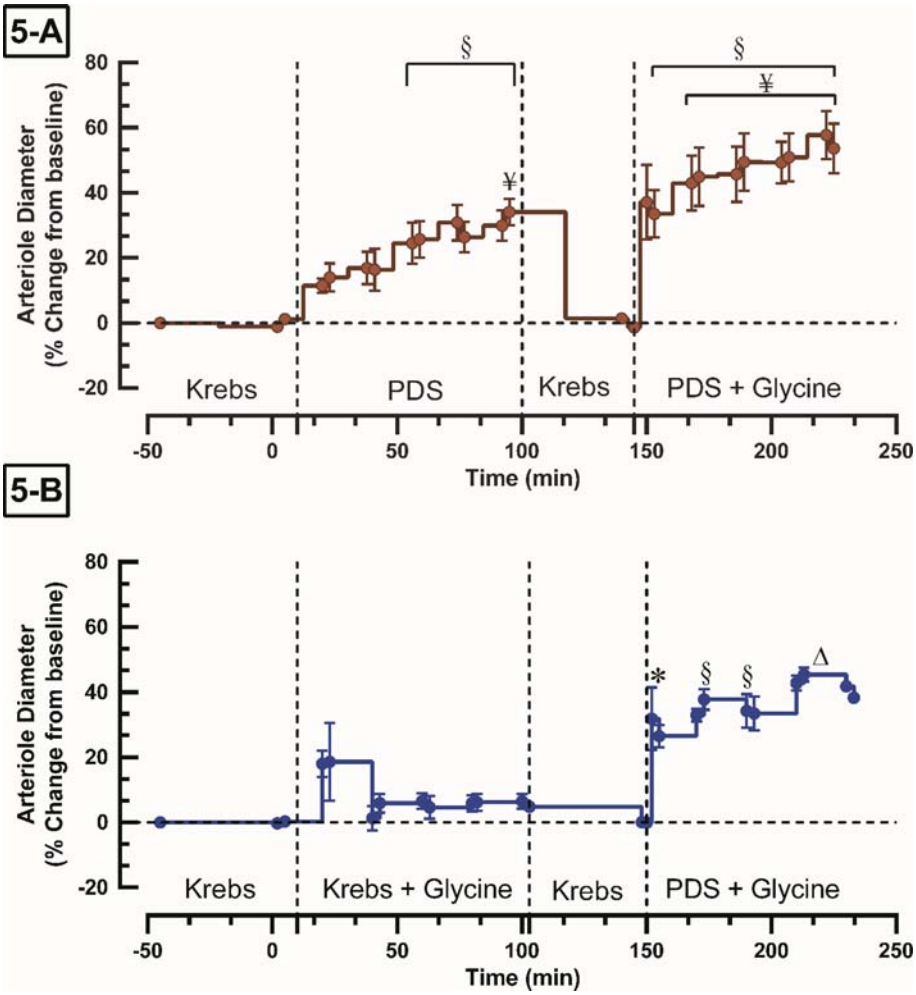

Figure 5. Third-order (A3) arteriolar reactivity. Series 1 (5A): Intestinal premucosal A3 arteriole (~15–20-μm diameter) response to peritoneal dialysis solution (PDS) and PDS supplemented with glycine (PDS + Glycine). Series 2 (5B): Intestinal premucosal A3 arteriole response to Krebs solution supplemented with glycine (Krebs + Glycine) and to PDS supplemented with glycine (PDS + Glycine). All diameter data were normalized and presented as percentage changes from baseline. PDS is a clinical peritoneal dialysis solution (Dianeal 2.27% dextrose solution). Krebs solution is a nonvasoactive salt-balanced solution containing no glucose. $^{*}p < 0.01$ versus baseline; $^{\S}p < 0.001$ and $^{\Delta}p < 0.0001$ versus Krebs + Glycine; $^{\S}p < 0.001$ and $^{¥}p < 0.0001$ versus PDS, by two-way analysis of variance and post Dunett test with correction for multiple comparisons.

occur, contact between intraperitoneal PDS and the anatomic peritoneal surface must be established.[1] During PD, this wetted peritoneal surface area is <50% of the actually available area of the anatomic peritoneum.[20] Maneuvers to increase the wetted surface by agitation or addition of a surfactant to PDS remarkably increase the fraction of the anatomic peritoneum in contact with intraperitoneal PDS. Recruitment of additional wetted surface area (unlike the use of vasodilators) is concurrently associated with enhanced solute clearance.[20–22] Carlsson and Rippe proposed that PDS-induced vasodilation is a plausible explanation for the observed increase in the mass transfer area coefficient of small solutes during the initial phase (2–15 minutes) of the dwell compared to the transport rate during the subsequent dwell time.[23] However, PDS-mediated dilation is not restricted to the initial phase of the dwell, and it is sustained for as long as the peritoneal tissue is in contact with PDS. Therefore, variations in small solute transport during the dwell time are also subjected to different concentration gradients during the dwell time. Although peritoneal functions and transport properties were not assessed in the present study, the decrease in the magnitude of PDS-induced peritoneal

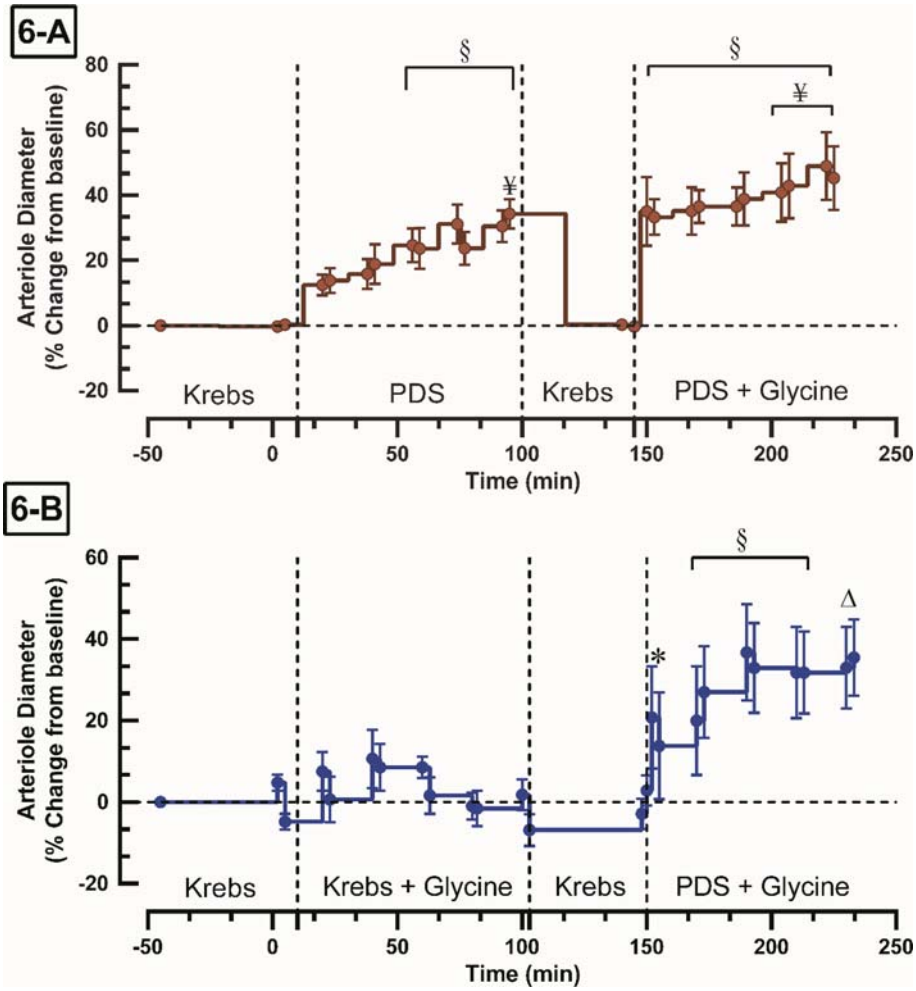

Figure 6. Fourth-order (A4) arteriolar reactivity. Series 1 (6A): Intestinal premucosal, precapillary A4 arteriole (~8–12-μm diameter) response to peritoneal dialysis solution (PDS) and PDS supplemented with glycine (PDS + Glycine). Series 2 (6B): Intestinal premucosal, precapillary A4 arteriole response Krebs solution supplemented with glycine (Krebs + Glycine) and to PDS supplemented with glycine (PDS + Glycine). All diameter data were normalized and presented as percentage changes from baseline. PDS is a clinical peritoneal dialysis solution (Dianeal 2.27% dextrose solution). Krebs solution is a nonvasoactive salt-balanced solution without glucose. $^{*}p < 0.01$ versus baseline; $^{§}p < 0.001$ and $^{Δ}p < 0.0001$ versus Krebs + Glycine; $^{§}p < 0.001$ and $^{¥}p < 0.0001$ versus PDS, by two-way analysis of variance and post Dunett test with correction for multiple comparisons.

microvascular vasodilation seen in elderly rats is unlikely to impact the peritoneal transport rates.

This study is the first to demonstrate the selective age-dependent decline of *in vivo* endothelial cell function in otherwise healthy animals. The study also demonstrated a protective role of glycine against aging-induced microvascular endothelial cell dysfunction. These findings were reproduced in an *in vivo* model of PDS-activated, microvascular endothelial-dependent dilation pathways in weaned, adult, and elderly rats. The microvascular endothelium is a highly metabolically active organ that maintains homeostasis through vital physiological processes at the molecular, cellular, and tissue levels.[24] At the tissue level, it acts as a selective barrier that regulates water and solute exchange between tissue, blood, and lymph. At the cellular level, it regulates the immune response through innate and adaptive mechanisms that allow leukocytes to transmigrate into the tissues.[24–26] It also maintains antithrombotic and prothrombotic dynamics to maintain homeostasis in healthy and diseased conditions.[24,26,27] Most importantly, the microvascular endothelium plays a critical role in the regulation of local vascular tone through the release of mediators of vasodilation and vasoconstriction in response to biochemical and physical stimuli.[24–29] The prevailing balance

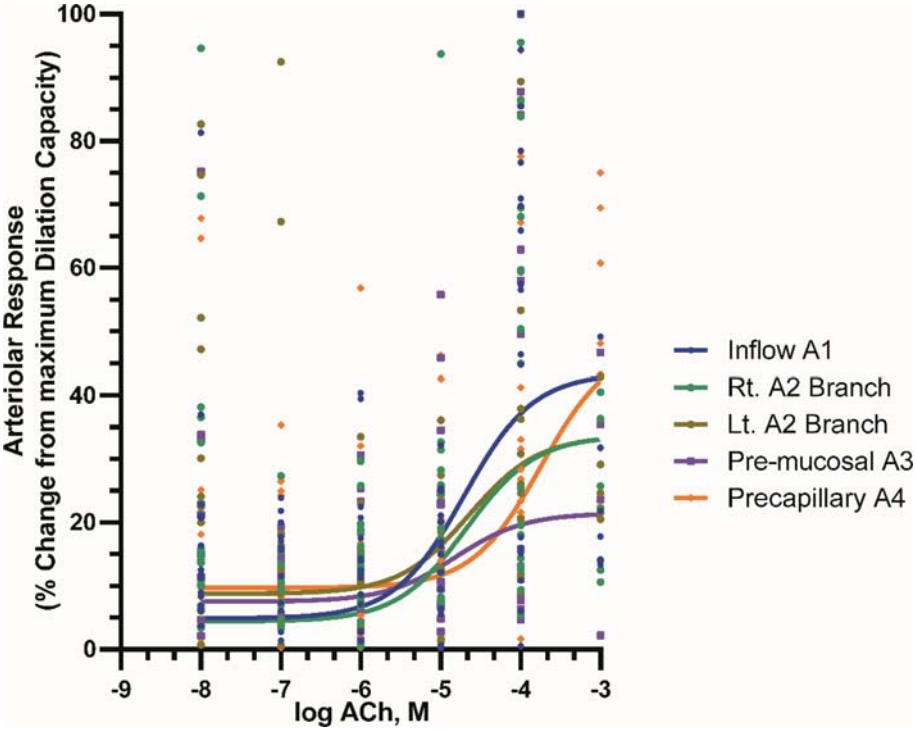

Figure 7. Experimental intestinal visceral arteriolar responses to cumulative acetylcholine concentrations. Inflow A1 = first order (A1) arteriole, Rt. A2 Branch = second order (A2) right branch arteriole, Lt. A2 Branch = second order (A2) left branch arteriole, premucosal A3 = third order (A3) arteriole, precapillary A4 = fourth order (A4) arteriole. Arteriolar responses were normalized as percentage changes from the maximum dilation capacity (obtained from a single dose of sodium nitroprusside, 10^{-4} M). Normalized arteriolar responses were plotted against the log concentration of acetylcholine (ACh) (Log 10^{-8} to 10^{-3} M). The best curve fit to the experimental data and the parameters defining the curves were determined by a computer-aided nonlinear regression analysis using the least-squares method.

	Inflow A1	Rt. A2 Branch	Lt. A2 Branch	Premucosal A3	Precapillary A4
Bottom	4.86 ± 3.43	4.41 ± 4.87	8.74 ± 3.91	7.50 ± 4.48	9.67 ± 2.36
Top	43.44 ± 7.53	33.66 ± 10.73	33.66 ± 8.78	21.45 ± 9.04	48.12 ± 12.36
LogEC50	−4.71 ± 0.36	−4.68 ± 0.69	−4.65 ± 0.66	−4.82 ± 1.22	−3.74 ± 0.36
Span	38.59 ± 7.75	29.25 ± 11.03	24.87 ± 8.99	24.87 ± 8.99	24.87 ± 12.28

between these mediators of dilation and constriction determines tissue perfusion.

Because of the complex and multifaceted functions of endothelial cells, a stimulus that is likely to alter all endothelial functions simultaneously, must have a magnitude that is extreme enough to cause damage rather than activation of the cells. In this context, aging-related microvascular endothelial cell dysfunction is more likely to be accelerated by disease conditions that are highly prevalent in the elderly population.[30] Comorbidities such as diabetes, hypertension, and dyslipidemia exert deleterious effects on the endothelial cells, leading to accelerated cell damage and death. Therefore, the association between aging-induced microvascular endothelial cell dysfunction and the accelerated endothelial damage induced by hypertension, diabetes, and dyslipidemia necessitates a comprehensive and individualized therapy combining exercise, nutritional support, vitamins, antioxidants, and other drugs to control the disease conditions commonly associated with old age.

Progressive decline in endothelial cell functions with age certainly contributes to the increased risk of cardiovascular diseases in the elderly.[31–34] This aging-related endothelial cell dysfunction is largely caused by increased vascular oxidative stress that results in increased reactive oxygen species (ROS) levels and attenuated endothelium-dependent dilator

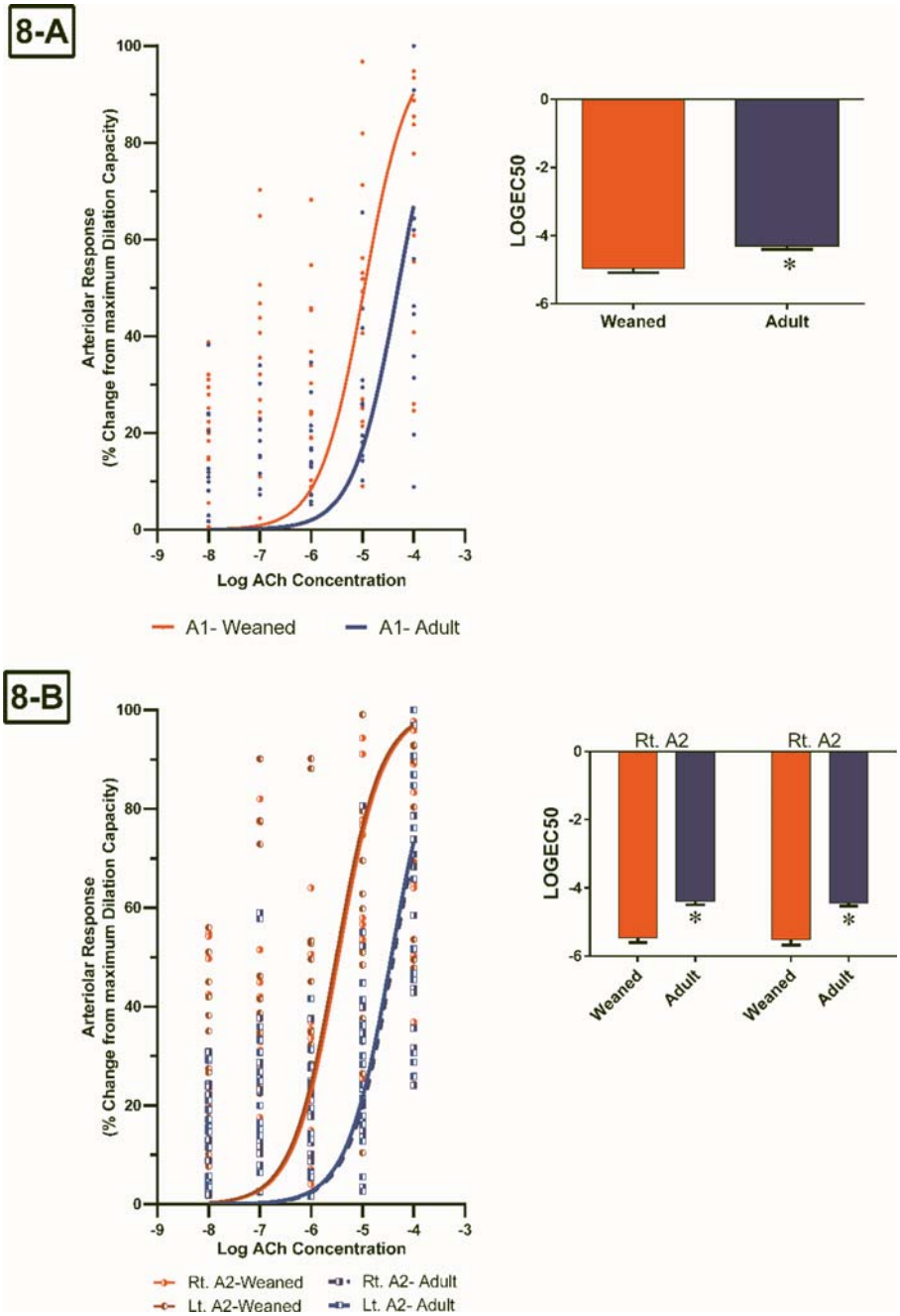

Figure 8. Experimental cumulative acetylcholine concentration – response curves (series 3 protocol) for first-order (A1) inflow arterioles (8A) and second-order (A2) arterioles (8B) in naïve weaned and adult rats. Arteriolar responses were normalized as percentage changes from the maximum arteriolar dilation capacity (obtained from a single dose of sodium nitroprusside, 10^{-4} M). Normalized arteriolar responses were plotted against the log concentration of acetylcholine (ACh) (Log 10^{-8} to 10^{-4} M). The best curve fit to the experimental data and the effective acetylcholine concentration that produced a 50% response (inset) are shown. The curves were determined by a computer-aided nonlinear regression analysis using the least-squares method. *$p < 0.01$, by unpaired t-test.

responses, which are largely mediated by the NO pathway.[35] At the microvascular endothelial cell level, increase in ROS levels with aging reduces NO bioavailability via the chemical inactivation of NO to form toxic peroxynitrite. This highly reactive nitrate isomer alters the endothelial NO synthase enzyme to become a superoxide-generating enzyme that further contributes to vascular oxidative stress. Because of this premise, recent research on aging has focused on the use of antioxidants to improve endothelial cell

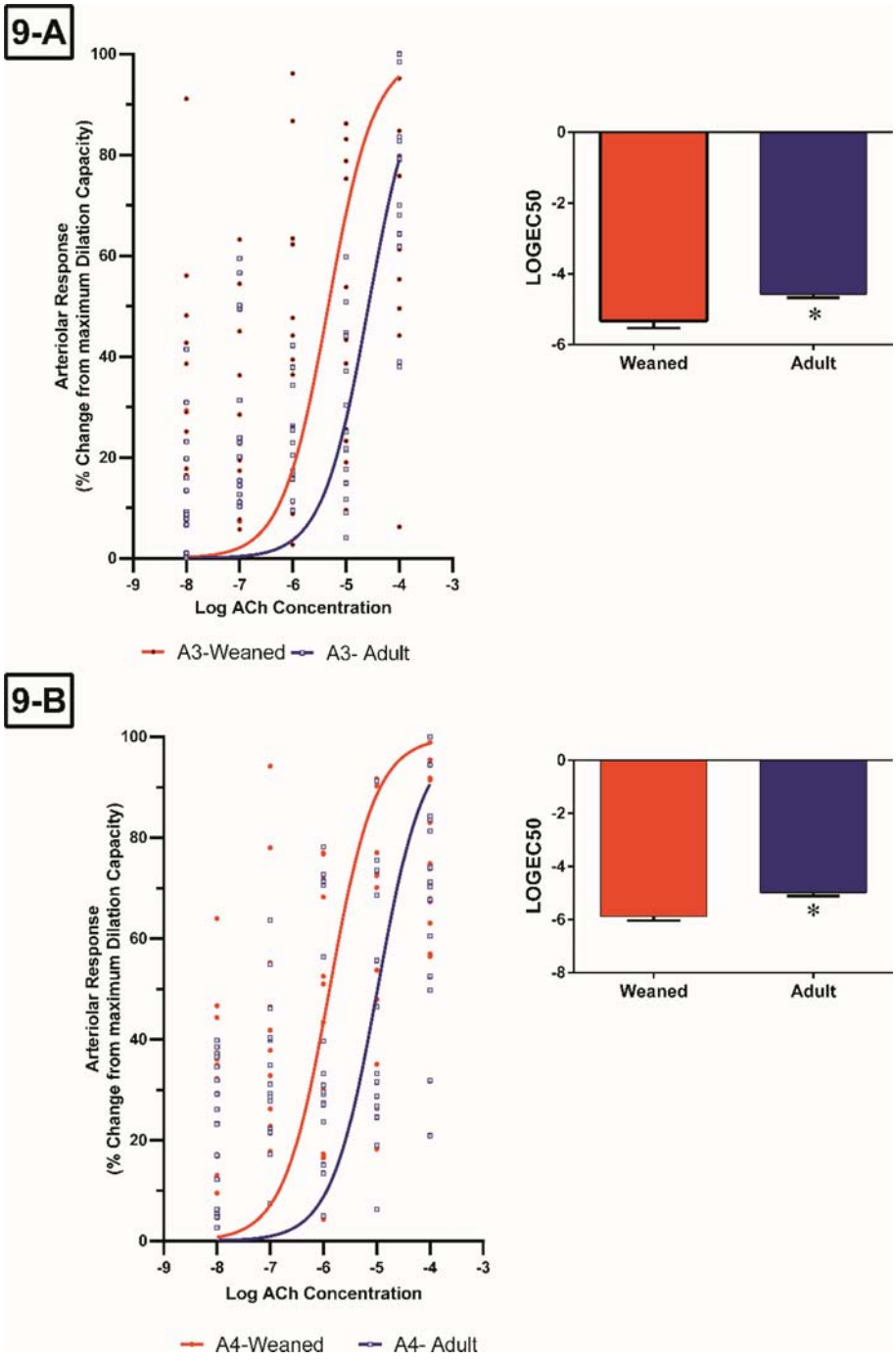

Figure 9. Experimental cumulative acetylcholine concentration–response curves (series 3 protocol) for third-order (A3) arterioles (9A) and fourth-order (A4) arterioles (9B) in naïve weaned and adult rats. Arteriolar responses were normalized as percentage changes from the maximum dilation capacity (obtained from a single dose of sodium nitroprusside, 10^{-4} M). Normalized arteriolar responses were plotted against the log concentration of acetylcholine (ACh) (Log 10^{-8} to 10^{-4} M). The best curve fit to the experimental data and the effective acetylcholine concentration that produced a 50% response (inset) are shown. The curves were determined by a computer-aided nonlinear regression analysis using the least-squares method. *$p < 0.01$, by unpaired t-test.

functions. Glycine is one of the natural antioxidants that also possesses antiaging properties. It has been shown that glycine improves age-related vascular endothelial cell dysfunction in elderly rats by improving NO bioavailability and age-associated mitochondrion respiration defects in human fibroblast cell lines via epigenetic mechanisms.[36,37] The present study verified that glycine alone, when added to the

Krebs solution in the tissue bath, causes a selective but transient minimal vasodilation of the smaller premucosal precapillary arterioles. However, the addition of glycine to PDS, in addition to enhancing the dilation effects of the solution, produces a pattern of progressive and time-dependent vasodilation at all microvascular levels. These vasoactive trends are explained by the acid–base properties of glycine. Glycine acts as a cation at a typical low pH of PDS and as an anion at a high pH of Krebs solution.[38] As the pH of PDS in the tissue bath continuously equilibrates over time with the blood, the acid–base properties of glycine also change.

CONCLUSION

The present study demonstrated ageing as an independent risk factor for microvascular endothelial cell dysfunction. This cellular dysfunction is manifested by the attenuation of endothelial-dependent, NO-mediated dilation in the elderly. Endothelial cell function in the elderly can be recovered by enforcing the bioavailability of endothelial-derived NO through glycine. Dietary glycine supplementation is a potential therapeutic strategy to decrease the burden of oxidative stress on the aged endothelium.

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Conflict of interest statement

The authors report no proprietary or financial interests in any product mentioned or concept discussed in this article.

Authors' contributions

E.Z. obtained the funding and designed the study. A.A, F.S., M.A., T.F., D.M., and E.Z. collected the data and conducted the literature search. B.J., M.H., M.Z., and E.Z. analyzed the data and critically evaluated the final draft. All authors participated in data interpretation and article preparation.

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