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Postnatal exposure to voluntary exercise but not the antioxidant catechin protects the vasculature after a switch to an atherogenic environment in middle-age mice

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Abstract

We aimed to evaluate the lasting functional imprinting of exercise (EX) and catechin (CAT) on the vascular function of middle-age mice switched to a proatherogenic environment. C57BL/6J mice (n=10–15 in each group) fed a regular diet (RD) were exposed from the age of 1 to 9 months either to EX (voluntary running; 2.7± 0.2 km/day), to the polyphenol CAT (30 mg/kg/day in drinking water), or to physical inactivity (PI). At 9 months of age, EX and CAT were stopped and mice either remained on the RD or were fed a Western diet (WD) for an additional 3 months. At 12 months of age, mice from all groups fed a WD had similar body mass, systolic blood pressure, and plasma total cholesterol, glucose, insulin, and isoprostane. Compared to the RD, the WD induced an indomethacin-sensitive aortic endothelium-dependent and independent dysfunction in PI mice (p<0.05) that was prevented by both EX and CAT; this benefit was associated with a higher (p< 0.05) non-nitric oxide/non-prostacyclin endothelium-dependent relaxation. While EX, but not PI or CAT, prevented vascular dysfunction induced by the WD in cerebral arteries, it had no effect in femoral arteries. The profiles of activity of antioxidant enzymes and of proinflammatory gene expression in the aorta suggest a better adaptation of EX>CAT>PI mice to stress. In conclusion, our data suggest that a postnatal exposure to EX, but not to CAT, imprints an adaptive defense capacity in the vasculature against a deleterious change in lifestyle.

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Keywords

Physical inactivity; Western diet; Endothelium; Antioxidant enzymes; Vascular smooth muscle cells

Introduction

Beyond genetic susceptibilities, the environment we live in is the main determinant of our health span [9]. It starts during the intrauterine environment that influences health outcome at adult age, as low birth weight is associated with an increased risk of diseases [2]. Epidemiological studies have clearly established that a lifelong healthy lifestyle, composed of moderate but regular physical exercise (EX) and a balanced diet, prolongs lifespan and reduces the odds of developing cardiovascular diseases (CVD) [10, 16, 45, 46, 59, 76]. The detrimental effects of the so-called Western diet (WD) and lifestyle on the cardiovascular system have, therefore, been established by numerous studies showing the contribution of physical inactivity (PI) and poor-quality/high-calorie intake on the development of obesity, hypertension, diabetes, and CVD [9, 37].

The primary target for stress induced by risk factors for CVD is the vascular endothelium. While not a medical condition, a chronic endothelial dysfunction is the initiating step of the atherosclerotic process [14, 73, 74]. Numerous preclinical studies have confirmed the cardiovascular benefits of EX on the endothelial function in aging mice [20], obese rats [7], and apoE$^{-/-}$ mice fed a high-fat diet [38]. In addition, the antioxidant polyphenol catechin (CAT) can prevent endothelial dysfunction in severely dyslipidemic mice if initiated early in life [19, 25]; we found, however, that chronic treatment with CAT was not effective and even deleterious if started in mice with established atherosclerosis [25], confirming the results of numerous clinical trials [66]. In contrast, the endothelial function of arteries from 1-year-old healthy C57BL/6 mice benefited more from CAT when initiated at the age of 9 months than when initiated at the age of 3 months [26]. Altogether, these data, in agreement with the clinical trials, suggest that (1) the timing of the treatment and (2) the environment (oxidative stress and inflammation) determine the endothelial protection given by antioxidants. It is unknown, however, if a healthy lifestyle, including EX, imprints the vascular endothelium, providing a better adaptive capacity to maintain its function against a deleterious and chronic change in environment.

In this study, we used two preventive interventions, voluntary EX and CAT, in young mice to test the hypothesis that these interventions will impose a unique molecular imprint to the endothelium, leading to a differential vascular reactivity after a switch in lifestyle at midlife. Our data demonstrate that lifelong PI combined or not with CAT limits the adaptive defense capacity of the vasculature compared to EX. They suggest, in agreement with our initial paradigm, that the magnitude of the age-dependent vascular dysfunction is directly related to its ability to limit the accumulation of damage.

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Methods

The procedures and protocols in our study were approved by the Montreal Heart Institute Animal Ethics Committee and performed in accordance with the *Guide for the Care and Use of Experimental Animals of the Canadian Council on Animal Care* and the *Guide for the Care and Use of Laboratory Animals* of the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Animals

C57BL/6J 1-month-old male mice (n=10–15 per group) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Animals were kept under standard conditions (24 °C; 12:12 h light/dark cycle). At 1 month of age, mice were randomly selected to perform voluntary EX, receive CAT or neither (PI) until the age of 9 months (Supplementary Fig. S1). EX mice had free access to a running wheel (Lafayette Instrument, Lafayette, IN, USA) without reward [5] and ran an average of 2.7±0.2 km/day over the 8-month period of exposure. The polyphenol CAT was dissolved in the drinking water to deliver a daily dose of 30 mg/kg previously shown to lower oxidative stress [18, 25], prevent endothelial dysfunction and arterial wall remodeling [6, 19, 24, 25], and improve cognition [18]. During that period, all mice were fed a regular diet (RD, 2018; Harlan Teklad Laboratories, Madison, WI, USA). At 9 months of age, EX and CAT were stopped. Mice were either sacrificed for study or randomly assigned to receive a 3-month RD or WD (88137; Harlan Teklad Laboratories, Madison, WI, USA) until the age of 12 months (Fig. S1). During the 11 months of experimentation, blood pressure was recorded every other week by tail plethysmography (Kent Scientific Corporation, Torrington, CT, USA) as described before [6]. Values of systolic blood pressure (SBP) presented are a mean of the measures obtained during the last three sessions of recording prior to sacrifice. Food consumption was measured in each group 1 week before the age of 9 and 12 months. We observed that EX mice ate less (p<0.05) at 9 months than PI mice (PI=4.0±0.1 g/day, EX=3.4±0.2 g/day, CAT=3.8± 0.1 g/day, n=9–10). Following the WD, EX and CAT mice ate less (p<0.05) than PI mice (PI=3.9±0.1 g/day, EX=3.3± 0.1 g/day, CAT=3.4±0.2 g/day, n=7–10). At 9 months of age, EX mice had no access to the running wheel at least 48 h before euthanasia to avoid an acute effect of EX. Mice were fasted overnight before their morning terminal anesthesia (44 mg/kg ketamine and 2.2 mg/kg xylazine). Blood was collected and plasma was frozen at −80 °C. Thoracic and abdominal aortas were harvested and placed in ice-cold physiological salt solution (PSS, pH 7.4; in millimoles per liter: 119 NaCl, 4.7 KCl, 1.18 KH_2PO_4, 1.17 $MgSO_4$, 24.9 $NaHCO_3$, 1.6 $CaCl_2$, 0.023 EDTA, and 10 glucose). Thoracic aortas were cut into 2-mm rings for vascular reactivity studies or embedded in optimal cutting temperature compound for immunostaining. Abdominal aortas were snap-frozen in liquid nitrogen and used for enzymatic activity and quantitative polymerase chain reaction (qPCR) studies. Femoral arteries were harvested and placed in ice-cold PSS to isolate a 2- to 3-mm-length arterial segments for endothelial function and compliance studies. Brain was removed from its cranial cavity and placed in ice-cold PSS to isolate posterior cerebral arteries (PCA) for endothelial function and compliance studies as previously reported [5].

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Plasma parameters

Cholesterol and glucose levels were measured at the Montreal Heart Institute Clinical Biochemistry Laboratory (Montreal, QC, Canada). 8-Iso-prostaglandin $F_{2\alpha}$ (isoprostane; Enzo Life Sciences, Farmingdale, NY, USA) and insulin (Alpco Diagnostics, Salem, NH, USA) were quantified using commercial kits and according to the manufacturers' protocols.

Endothelial function in aortas

Rings of 2 mm isolated from the thoracic aorta were mounted on 20-μm tungsten wires in microvessel myographs (IMF, University of Vermont, Burlington, VT, USA) as previously described [70]. Vessels were stretched to obtain an optimal basal tension of 1.5 g as determined in preliminary studies; no differences in basal tension were observed between groups (data not included). The viability of the arterial rings was tested with a 40-mM KCl-PSS solution. After two washout periods, the vessels were equilibrated for 30–45 min alone, in the presence of Nω-nitro-L-arginine (L-NNA, 100 μM, a nitric oxide synthase [NOS] inhibitor), indomethacin (10 μM, a nonselective cyclooxygenase inhibitor), or with both L-NNA and indomethacin to study non-nitric oxide (NO)/non-prostanoid-dependent relaxations. First, a concentration–response curve with the thromboxane A_2 analog 9,11-dideoxy-11α,9α-ep-oxy-methano-prostaglandin $F_{2\alpha}$ (U46619, 0.1 nM to 10 μM) was obtained for each group (n=3–5, in duplicate) to measure the half-maximum effective concentration (EC_{50}) (Table S1). In subsequent experiments (n = 3–9 per group), aorta segments were preconstricted at the EC_{50} and relaxation curves to acetylcholine (Ach, 0.1 nM to 30 μM) and sodium nitroprusside (SNP, 0.1 nM to 30 μM) were performed. Two consecutive concentration–response curves separated by 30–45 min washout periods were obtained from each aortic segment. Preliminary studies showed no effect of consecutive random concentration–response curves on relaxation (data not included). The maximal contraction was obtained at the end of the protocol by adding 127 mM KCl-PSS.

Reverse transcriptase quantitative polymerase chain reaction

Total RNA extraction was performed with the RNeasy Mini Kit (Qiagen Canada, Toronto, ON, Canada) following the manufacturer's protocol. Reverse transcriptase reaction (100 ng total RNA) was performed as described previously [24] using the Moloney murine leukemia virus reverse transcriptase (200 U; Invitrogen, Carlsbad, CA, USA). qPCR were performed with platinum SYBR Green qPCR SuperMix-UDG (Invitrogen, Carlsbad, CA, USA). Primers were selected in two different exons spanning a large intronic sequence to avoid amplification of genomic DNA (Table S2). Annealing and elongation temperatures, cDNA template quantity, and primer concentrations were optimized for each pair of primers to generate a standard curve with an efficacy of 100±10 %. Cyclophilin A was used as a normalizer and relative gene expression was calculated by the ΔΔCT method [53].

Antioxidant enzyme activity in aortas

Abdominal aortas were disrupted with a mortar and pestle on dry ice and kept at −80 °C. The powder was manually homogenized in either 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.2; in millimoles per liter: 20 HEPES, 1 ethylene glycol tetraacetic acid [EGTA], 210 mannitol, and 70 sucrose) for catalase and superoxide

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dismutase 2 (SOD2) assays or Tris–HCl buffer (pH 7.5; in millimoles per liter: 50 Tris–HCl, 5 EDTA, and 1 DTT) for glutathione peroxidase (GPx) assay. Samples homogenized in the HEPES buffer were split into two tubes and centrifuged 5 min at 1,500×*g* (4 °C) for SOD2 assay or 15 min at 10,000×*g* (4 °C) for catalase activity. Samples for GPx assay were centrifuged for 15 min at 10,000×*g* (4 °C). An aliquot of each sample was kept to measure protein concentration (Bradford assay). Further steps were performed as recommended by the manufacturer's protocol (Cayman Chemical Company, Ann Arbor, MI, USA). SOD2 activity was determined in the presence of 1 mM of potassium cyanide to block copper–zinc SOD activities [62]. Activity values (in units) were adjusted with protein content.

Endothelial function in femoral arteries

Segments of the left or right gracilis artery were dissected in ice-cold PSS to remove surrounding fat and tissues [35]. A 2- to 3-mm-length arterial segment was isolated, cannulated at both ends, and pressurized at 80 mmHg in no flow condition in a pressure myograph. Internal pressure was maintained constant and real-time diameter changes were monitored using a pressure servo-control and a video dimension analyzer, respectively (Living System, Burlington, VT, USA). All experiments were conducted on segments with an internal diameter of 175–210 μM when pressurized at 80 mmHg. An equilibration time of 45 min was allowed before starting the experiment. A single cumulative concentration–response curve to Ach (1 nM to 30 μM) was obtained in vessel preconstricted with phenylephrine (1–30 μM). L-NNA, indomethacin, or a combination of both drugs was added during the equilibration period.

Endothelial function in cerebral arteries

PCAwere isolated, cannulated at both ends, and pressurized as previously described [17]. Flow-mediated dilations (FMD) were induced on phenylephrine (30 μM) preconstricted arteries [6]. Arteries were perfused with PSS and aerated with 12 % O_2/5 % CO_2/83 % N_2. A single cumulative curve (from 0 to 20 dyn/cm^2, with 2 dyn/cm^2 steps between 0 and 10 dyn/cm^2 followed by two 5 dyn/cm^2 steps, at constant pressure of 60 mmHg) was performed on each segment [5]. The flow rate through the lumen (Q; in milliliters per second) required to match a given shear stress value (τ; in dynes per square centimeter) was calculated for each point on the curve according to $Q=\tau\pi r^3/4\eta$, where η represents the viscosity (0.009 Poise) and r is the inside radius (in centimeters). The applied calculated shear stress was in the physiological range (≈0–25 dyn/cm^2) [42, 50]. The data are presented as the percentage of maximal dilation for every shear stress value.

Compliance studies in femoral and cerebral arteries

Femoral arteries and PCA were used for the in vitro assessment of compliance, measured in passive conditions to reflect mechanical properties of the vascular wall [5, 6]. Passive pressure–diameter curves were conducted in a Ca^{2+}-free PSS containing 1 mM of EGTA and 10 μM of SNP in order to abolish myogenic tone and to solely assess the mechanical properties of the arteries. Phenylephrine (10 μM) never induced a reduction in diameter in these conditions (data not shown). Lumen diameter and outer diameter changes were measured after each increment in the intraluminal pressure (from 10 to 120 mmHg, with a first 10-mmHg step followed by a 20-mmHg step) in order to calculate mechanical

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parameters of the arterial wall. The circumferential wall strain (in percent) was calculated according to $[(D-D_{10\ mmHg})/D_{10\ mmHg}]$, where D is the diameter at a given pressure and $D_{10\ mmHg}$ is the initial diameter at the initial pressure. The circumferential wall stress (in dynes per square centimeter) was calculated according to $[(P\times1{,}334\times D/2)\times$wall thickness$]$, where P is the given intraluminal pressure (1 mmHg= 1,334 dyn/cm^2) and wall thickness (in micromolars) is calculated with [(external diameter–lumen diameter)/2]. The stress–strain relationship was calculated by fitting the data to a nonlinear exponential curve ($\text{stress}=\text{stress}_0\beta^{\text{strain}}$), where stress_0 is the value when strain equals 0 and β, is a constant representing Young's elastic modulus (YEM).

Statistical analyses

All groups were composed of 10 mice, except for CAT of 12 months fed RD or WD (n=15). The n refers to the number of animals used in each protocol. A study number of four animals per group of experiments has 90 % power to detect a 30 % absolute variation in vascular reactivity, the most variable parameter measured in this study assessed during preliminary studies. All protocols, including reactivity studies, were performed in duplicate and averaged before individual data inclusion for statistical analysis. Results are presented as the mean ±standard error of the mean (SEM). EC_{50} and the pD_2 value, the negative log of the EC_{50}, were measured from individual concentration–response curves. Two-way analysis of variance (ANOVA) was used to test for interactions and main effects between initial treatments and age/diet of mice. When interactions were not statistically significant, marginal means were compared followed by the Bonferroni approach for multiple comparisons. When interactions were statistically significant, one-way ANOVA was used to compare cell means followed by the Bonferroni approach. For analysis of maximal relaxations (E_{max}) in aorta and femoral arteries, Dunnett's approach was used instead of the Bonferroni approach. Usual assumptions such as normality and homoscedasticity of the variance were checked. When variances were unequal, usual transformations, such as logarithmic, square root, or inverse function, were used. If unequal variances remained after transformation, covariance pattern models were used. When normality was absent even after transformations, the Kruskal–Wallis test was used followed by the Mann–Whitney pairwise comparisons with adjusted p values. SPSS (IBM Corporation, Armonk, NY, USA) and SAS (SAS Institute, Cary, NC, USA) softwares were used for the statistical analyses. A value of $p<0.05$ was considered statistically significant.

Results

At the end of the preventive interventions, mice from the three groups had a similar body mass, SBP, as well as plasma glucose, insulin, and total cholesterol (TC) levels (Table 1). Glycemia was high at 9 months of age in all groups (Table 1) compared to 3-month-old mice [5]; this age-dependent increase in circulating glucose has been reported in rat [55], monkey [41], and human [3].

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Effect on endothelium-dependent and endothelium-independent function of EX, CAT, and PI at 9 months

Maximal relaxations to Ach and SNP in aortas and to Ach in femoral arteries were similar in all groups (Fig. 1a, d; Tables S3 and S4), with no marked differences in sensitivity (data not included). In aortas isolated from PI and CAT mice, indomethacin (nonspecific COX1/2 inhibitor) significantly enhanced the maximal relaxation to Ach (Table S3). At 20 dyn/cm^2, the highest shear stress imposed, FMD of cerebral arteries isolated from PI and CAT mice were lower than in arteries isolated from EX mice (Fig. 1g).

Antioxidant enzymatic activities at 9 months

Catalase activity was higher in PI mice (Fig. 2a), without change in SOD2 and GPx activities (Fig. 2d, g). Systemic oxidative stress was evaluated by the levels of isoprostane in the plasma, a marker of lipid peroxidation [30]. Surprisingly, CAT-treated mice expressed higher levels of isoprostane in the blood than EX mice (Fig. 2j); aortic oxidative stress measured with the florescent probe dihydroethidium, however, was equivalent in all groups (data not included).

Impact of the switch in lifestyle

At 12 months of age, mouse physiological and plasma parameters remained stable when switched to PI and a RD (Table 1). In all mice switched to a WD, however, body mass, glucose, insulin, and cholesterol increased (Table 1). No atherosclerotic plaques were visible in aortas.

Endothelium-dependent and endothelium-independent function 3 months after the switch of lifestyle

The maximal relaxant response induced by Ach in aortas isolated from PI mice was significantly reduced by the WD compared to the RD and abolished in the presence of L-NNA (Fig. 1b, c; Table S3); indomethacin, however, fully restored Ach-induced maximal relaxation compared to aorta segments isolated from the two other groups of mice (Table S3). These results, therefore, suggest (1) a reduction in NO production and (2) the presence of an antirelaxant constricting prostanoid, both limiting Ach-induced endothelium-dependent relaxation. In contrast to PI mice, the relaxation of aorta segments isolated from EX and CAT mice fed a WD was maintained at 12 months of age (Fig. 1c; Table S3). Indomethacin did not alter the relaxation induced by Ach, while L-NNA reduced it by ~60 % (Table S3). After combined NOS and COX1/2 inhibition, however, the relaxation induced by Ach was lower in PI mice compared to EX and CAT mice (Fig. S2a; Table S3), demonstrating that previous exposure to EX and CAT increased the contribution of a compensatory non-NO/non-prostacyclin (PGI_2) relaxant factor while likely maintaining NO production. Similarly to Ach, the relaxation induced by SNP was lower in the aorta isolated from 12-month-old PI mice fed a WD compared to all other groups (Table S3), suggesting either an impairment of vascular smooth muscle cells (VSMC) to SNP-derived NO or, more likely, that the COX1/2 derivative limits as well the endothelium-independent SNP-induced VSMC relaxation.

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The relaxation of the femoral arteries was not altered at 12 months in the three groups (Fig. 1e, f; Table S4). Nonetheless, differences in the contribution of NOS and COX1/2 activities were noted: individual inhibition of NOS and COX1/2 both reduced the relaxation induced by Ach in PI mice independently of the diet (Table S4). In contrast, the combination of L-NNA and indomethacin strongly reduced the relaxation induced by Ach in femoral arteries isolated from PI mice and from CAT mice fed a WD, but not an RD (Table S4). L-NNA and indomethacin, combined or not, did not significantly affect the relaxation of femoral arteries isolated from 12-month-old EX mice (Table S4).

Cerebral arteries isolated from EX mice and pressurized at 60 mmHg had a consistent greater FMD at 9 months (Fig. 1g) and at 12 months after 3 months of RD (Fig. 1h) or of WD (Fig. 1i). CAT neither prevented the age-related decline in FMD nor the deleterious impact of the WD on FMD (Fig. 1h, i). The WD, however, did not magnify the decline in endothelial function associated with a lifelong PI when fed an RD (Fig. 1h, i).

Antioxidant enzymatic activities and inflammatory genes expression 3 months after the switch of lifestyle

After 3 months of exposure to the WD, SOD2 activity was lower in EX mice only (Fig. 2f). Catalase and GPx activities did not increase with the WD compared to that measured at 9 months (Fig. 2c, i), but it increased in CAT mice after the RD (Fig. 2b). The isoprostane levels remained elevated in CAT-treated mice, while the WD increased isoprostane in PI and EX mice to become similar in all groups (Fig. 2l). No change in aortic oxidative stress was observed (data not included). Finally, the expression of the inflammation-related genes COX2 and angiopoietin-like 2 (angptl2) increased in CAT-treated mice only following the WD (Table S5).

Impact of the interventions on the femoral and cerebral artery biomechanics

The biomechanical behavior of the wall of conductance and muscular arteries influences blood flow dynamics and can be modified by a change in endothelial function and EX training [5, 48]. At 9 months, femoral arteries isolated from EX mice were more compliant compared with PI and CAT mice, evidenced by a rightward shift of the passive stress–strain curve (Fig. 3a) and a lower YEM (Table S6). The compliance remained greater at 12 months of age in femoral arteries isolated from EX mice compared to those of PI mice, but nonetheless stiffened 3 months after preventing access to the EX wheel and exposure to either diets (Fig. 3c, e; Table S6). In contrast, removal of CAT at 9 months led to an increase in compliance at 12 months, leading to a similar level of compliance in femoral arteries isolated from CAT and EX mice with either diets (Fig. 3c, e; Table S6). PI, alone or combined with a WD, did not modify the compliance of the femoral artery (Fig. 3c, e; Table S6).

In contrast to the femoral artery, the compliance of mouse cerebral arteries is paradoxically increased by endothelial dysfunction [5, 6]. At 9 months of age, the compliance of the cerebral arteries isolated from CAT mice was greater compared to the two other groups (Fig. 3b), although the YEM was not changed significantly (Table S6), suggesting that CAT induced adverse remodeling of the cerebrovascular wall. At 12 months, the absence of CAT

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normalized the deleterious effect of CAT pretreatment on the compliance of the cerebral artery (Fig. 3d, f; Table S6). As expected, the WD shifted to the right the passive stress–strain curve in the cerebral arteries isolated from PI mice (Fig. 3f), together with a reduction of the YEM compared to EX mice (Table S6). These data demonstrate that the WD induced a weakening and a degradation of the biomechanical properties of the wall of the cerebral arteries in PI and CAT mice, while EX mice were protected.

Discussion

Vascular imprinting

The major finding of this study is that two interventions initiated at a very early age and known to be protective of the cardiovascular system imprint the vasculature so that it responds differentially in the face of a deleterious change of environment at middle age. In our experimental paradigm, early exposure to EX better protected the vasculature than CAT. This demonstrates that a healthy lifestyle made of voluntary EX and a balanced diet during development and maturation has a lasting protective impact on the vascular function. More importantly, our data reveal that the animals previously treated with CAT responded poorly to the WD with more evidence of inflammation and cerebrovascular adverse remodeling, making them at risk, although differently from PI animals, when transposed to a deleterious environment.

It is not well-known if the beneficial effects of a healthy lifestyle on the cardiovascular system would outlast a switch to a deleterious environment. One study reported that 3 weeks of EX was sufficient to prevent the development of obesity induced by a high-fat diet 10 weeks later [52]; yet, another reported that cessation of EX predisposed rats to hepatic steatosis [60]. By opposition, a short-term period of forced sedentary behavior (5 to 7 days) has been shown to be sufficient to alter vascular function [31, 43]. Our study is, therefore, unique because it reveals that voluntary EX during the first 9 months of life permitted the maintenance of the vascular (both endothelium-dependent and endothelium-independent) function, the optimal biomechanics of the cerebral and femoral arteries, together with a low SOD2 activity and low expression of inflammatory markers. These results suggest that EX mice were biologically *equipped* to face a metabolic stress, at least for 3 months.

EX facilitates adaptation

EX is known to stimulate endothelial NOS activity and the expression of antioxidant enzymes [27, 58]. Yet, EX is also known to transiently increase oxidative stress, and the beneficial adaptive changes can be prevented by a concomitant intake of antioxidants [29, 63]. Reactive oxygen species signaling is essential to regulate cellular function [28, 61], including to signal for adaptation to EX [23], and their inactivation at critical time points is deleterious [26, 29, 63]. This may explain why CAT alone, a potent antioxidant, neither fully preserved the vascular function nor the biomechanical properties compared to PI mice and even increased the expression of inflammatory markers and isoprostane. Hence, the impact of the two preventive approaches on the cardiovascular function diverged after 3 months of exposure to PI combined with a WD despite a similar increase in plasma TC, glucose, and insulin and in body mass (Table 1) that are associated with increased oxidative stress (Fig.

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2l) and overall risk of CVD [39]. The lasting impact or *memory* of the biological molecular modeling induced by EX is, therefore, reflected by the overexpression of stress resistance pathways [40, 56–58] and might explain the better outcome in EX mice.

Adaptive mechanisms

We previously reported that dyslipidemia stimulated the expression of an adaptive NO-independent endothelial pathway in mouse femoral arteries [36] that had been identified as endothelium-derived hyperpolarizing factor in mesenteric arteries by others [11, 78]. The impairment of the NO pathway seen in inactive aging mice [38, 65] has been shown to be prevented by EX or an antioxidant treatment [20, 79]. Interestingly, EX permitted the maintenance of a better vascular function in cerebral arteries than CAT in all conditions, demonstrating that EX is more potent at protecting the cerebral circulation than a single antioxidant intervention. Our data are the first to demonstrate that only EX leads to a lasting protection of the vasculature against a change in environment.

Role of oxidative stress

Additional mechanisms are likely to contribute to the beneficial effects of EX. Aging is associated with an increase in oxidative damages associated with a decrease in antioxidant mechanisms [28, 32] accelerated by a WD [13]. The higher SOD2 activity in PI and CAT mice compared to EX mice following the WD suggests a higher production of superoxide anion in these two groups. Superoxide can originate from different vascular sources: NADPH oxidase, xanthine oxidase, COX1/2, and uncoupled NOS [8, 71]. Aging [14], PI [38], and dyslipidemia [19] can induce oxidative damage by the generation of superoxide anion that will impair NO and PGI_2 relaxations [71]; EX can restore endothelial function and lower NADPH oxidase activity in the aorta of very old mice [20]. We reported that CAT reduces NADPH oxidase activity in cerebral arteries from 1-year-old atherosclerotic mice [19]. Excess of H_2O_2 can as well be deleterious for the vasculature and promote atherosclerosis [77]. At low levels of H_2O_2, GPx and peroxiredoxin prevent H_2O_2-related damage from normal metabolic activity; in cultured cells, however, an increase or excess of H_2O_2 inactivates GPx and peroxiredoxin, forcing catalase to compensate [44]. The higher level of catalase activity in PI mice at 9 months of age and in CAT mice fed a RD at 12 months of age suggests a greater load of H_2O_2 in these two groups. In CAT-treated mice, cessation of CAT at 9 months of age is likely responsible for the rise in catalase activity as an adaptive reaction to the reduction in antioxidant load. Surprisingly, however, the WD prevented this adaptive rise in catalase activity in CAT mice. The dominant, although likely necessary, role of antioxidant enzymes is, therefore, uncertain. Studies showed that EX prevented the decline in antioxidant enzymes function or expression [20, 51, 54], while others have reported no direct effect on SOD, catalase, or GPx activity or expression despite lower oxidative damage or increased NO bioavailability [4, 7, 22]. Similarly, antioxidant treatments are effective to prevent the decrease in anti-oxidant function in pro-inflammatory and pro-oxidant conditions, but cannot improve antioxidant enzyme profiles [1, 49]. In these healthy mice with no genetically augmented susceptibility to develop endothelial dysfunction and atherosclerosis, the WD neither produced a major global decline in antioxidant enzyme activity nor an increase in aortic oxidative stress: yet, PI induced a major reduction in vascular function in the aorta.

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Inflammation

Low-grade inflammation is a hallmark of endothelial dysfunction [72]. The WD increased COX2 and angptl2 expression, two markers of inflammation, in CAT mice only. We previously reported that COX2 expression correlated with the level of damage in endothelial cells isolated from coronary artery disease patients [75], while others showed that oxidative stress stimulated COX2 expression [21]. In addition, we reported that 9 months of CAT magnified 50-folds the rise in COX2 expression associated with aging in the mouse aorta [26]. Angptl2, on the other hand, is associated with inflammation as its presence is increased in patients with rheumatoid arthritis and abdominal aortic aneurysm [47, 69], while its deletion can lower pro-inflammatory cytokines in rodents [67, 69]. Hence, our results suggest that early CAT treatment induces a pro-inflammatory environment upon the challenge of a WD: we propose that CAT limited the development of antioxidant defense mechanisms able to counteract the stress induced by the WD.

Endothelium-independent functions

Despite numerous studies showing no influence of cardiovascular risk factor on endothelium-independent relaxation [14, 20, 38, 51], the relaxation induced by SNP was markedly reduced in aortas isolated from PI mice fed a WD. Aging alters both the endothelium and the VSMC [34], and endothelium-independent response can be altered in presence of hyperinsulinemia in patients with metabolic syndrome [64] or by acute hyperhomocysteinemia in diabetic patients [12]. On the other hand, EX has been shown to increase the relaxant response to NO donors in humans and rabbits [15, 33], demonstrating that it can reverse and/or counteract the dysfunctional VSMC relaxant capacities. The mechanism involved in the impaired SNP response remains elusive; nonetheless, the equally reduced endothelium-dependent relaxation to Ach was prevented in the presence of indomethacin. Therefore, the default of relaxation is reversible and we can speculate that constricting COX1/2 derivatives may also counteract the VSMC relaxation to SNP. Altogether, our data suggest that an efficient vascular relaxation depends on a fine balance between relaxing and constricting factors highly sensitive to the environment.

Limitations

Our study has limitations. The statistical determination of the number of animals was set to only detect significant (>30 %) changes in functions between groups. This may have decreased the power to detect fine changes in functions that may be significant and could widen in later life. The design of the experiments is complex and the parameters are numerous; this study needs to be replicated to address specifically the modifications induced by an abrupt change in the environment, an approach that would increase the power to detect biological modifications, notably the role of COX1/2 derivatives on the vascular relaxation. We believe, however, that this design reflects a clinical reality associated to increasing prevalence in the migration of population with the consequent changes in lifestyles, a phenomenon restricted in the past to “Westernized” isolated societies [68].

In conclusion, we demonstrate that a healthy lifestyle at a young age including voluntary EX and a balanced diet, but not a single antioxidant, is protective at middle age when challenged

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by an environmental stress, likely by stimulating the expression of stress resistance pathways.

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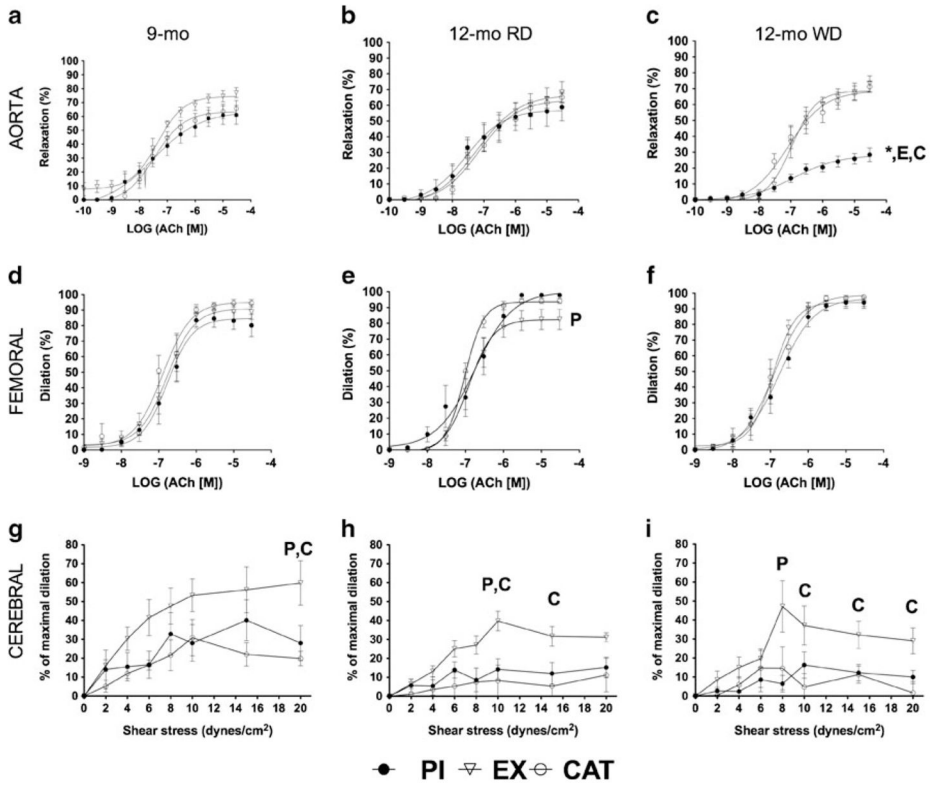

Fig. 1.

Main effects on vascular function of a switch from PI, EX (voluntary wheel running), or CAT (30 mg/kg/day in drinking water) with a RD from the age of 1 to 9 months to PI and a WD from the age of 9 to 12 months. Ach induced endothelium-dependent relaxation of the aorta (**a**, **b**, **c**; n=4–9) and femoral arteries (**d**, **e**, **f**; n=5–9) and flow-mediated endothelium-dependent dilation of cerebral arteries (**g**, **h**, **i**; n=6–10). Results are presented as the mean ±SEM. *p<0.05 compared to 12 months of age with RD (treatment matching group), ^{P}p<0.05 compared to PI, ^{E}p<0.05 compared to EX, and ^{C}p<0.05 compared to CAT

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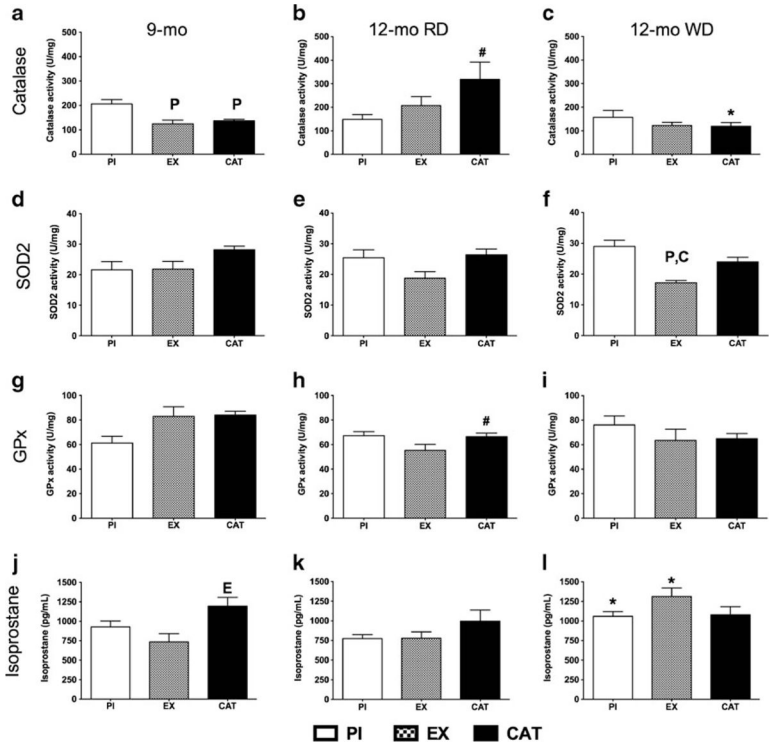

Fig. 2.

Effects on antioxidant activity of a switch from PI, EX (voluntary wheel running), or CAT (30 mg/kg/day in drinking water) with a RD from the age of 1 to 9 months to PI and a WD from the age of 9 to 12 months. Oxidative stress was evaluated by quantifying in the aorta the activity of catalase (**a**, **b**, **c**; n=4–5), SOD2 (**d**, **e**, **f**; n=4–5), and GPx (**g**, **h**, **i**; n=4–6) and by measuring the circulating levels of isoprostane (**j**, **k**, **l**; n=3–5). Results are presented as the mean± SEM. $^{\#}p<0.05$ compared to 9 months of age (treatment matching group), $^{*}p<0.05$ compared to 12 months of age with RD (treatment matching group), $^{P}p<0.05$ compared to PI, $^{E}p<0.05$ compared to EX, and $^{C}p<0.05$ compared to CAT

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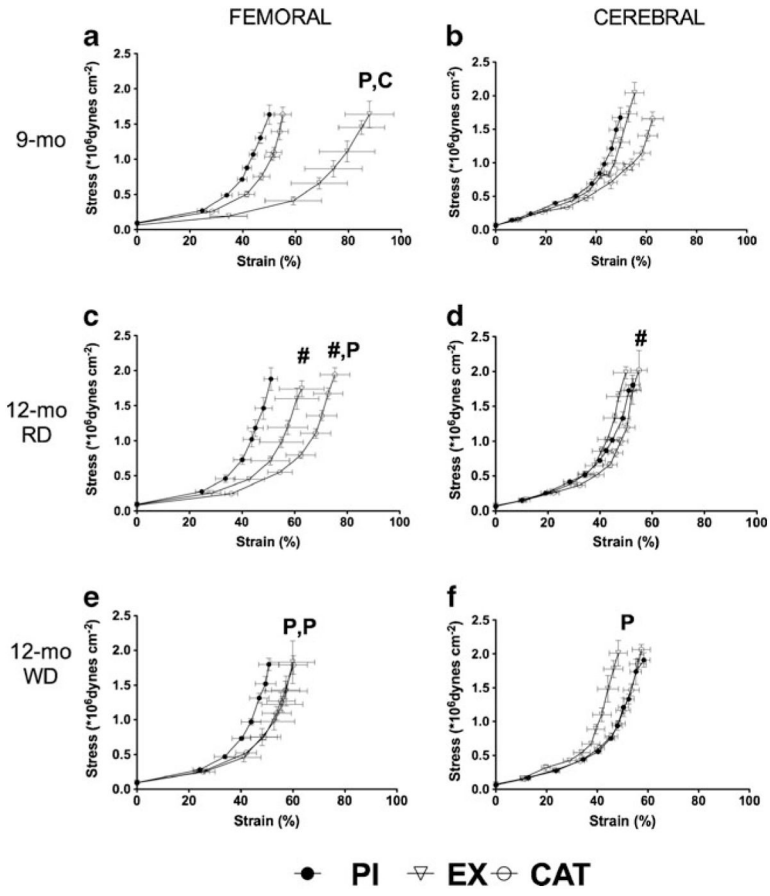

Fig. 3.

Major effects on the structure/function relationship (assessed by measuring compliance) of a switch from PI, EX (voluntary wheel running), or CAT (30 mg/kg/day in drinking water) with a RD from the age of 1 to 9 months to PI and a WD from the age of 9 to 12 months. Compliance was measured in the femoral (**a**, **c**, **e**; n=5–9) and the cerebral arteries (**b**, **d**, **f**; n=6–10). Results are presented as the mean±SEM. $^{\#}p<0.05$ compared to 9 months of age (treatment matching group), $^{P}p<0.05$ compared to PI, and $^{C}p<0.05$ compared to CAT

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Table 1

Body mass, plasma glucose, insulin, total cholesterol (TC), and SBP from PI, EX (voluntary wheel running), and CAT-treated (30 mg/kg/day in drinking water) mice from 1 to 9 months of age fed with a RD (9 months) and then maintained for 3 months in inactive conditions and fed either a RD (12 months) or a WD (12 months)

Age/diets	**9 months RD**			**12 months RD**			**12 months WD**			
Treatments	**PI**	**EX**	**CAT**	**PI**	**EX**	**CAT**	**PI**	**EX**	**CAT**	***n***
Body mass (g)	30.4±1.3	32.4±1.2	31.1±1.2	33.4±1.6	35.7±1.9	31.0±0.8	44.9±1.5 *	43.9±1.6 *	45.5±0.9 *	9–15
Glucose (mmol/L)	17.5±1.1	15.9±1.7	19.2±0.8	16.4±0.9	20.0±1.9	15.7±0.8	22.3±0.8 *	19.5±2.6	20.5±1.9 *	4–7
Insulin (ng/ml)	0.17±0.04	0.27±0.06	0.19±0.06	0.29±0.09	0.23±0.08	0.21±0.05	0.49±0.12 *	0.57±0.18 *	0.44±0.11 *	8–10
TC (mmol/L)	2.4±0.1	2.8±0.1	2.8±0.1	2.6±0.1	2.6±0.3	2.9±0.1	6.9±1.1 *	7.2±1.1 *	5.4±0.6 *	4–7
SBP (mmHg)	141.0±6.2	142.3±2.9	148.1±2.8	143.3±5.5	140.6±3.7	149.0±1.8	134.1±8.1	145.5±4.3	138.8±3.2	4–14

Results are presented as the mean±SEM

* $p<0.05$ compared to 12 months RD (treatment matching group)