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Western-type diet induces senescence, modifies vascular function in non-senescence mice and triggers adaptive mechanisms in senescent ones

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ABSTRACT

The effects of high-fat diet ingestion on senescence-induced modulation of contractile responses to phenylephrine (Phe) were determined in aortas of senescence-accelerated (SAMP8) and non-senescent (SAMR1) mice fed (8 weeks) a Western-type high-fat diet (WD). Increased levels of senescence-associated β-galactosidase staining were found in aortas of SAMP8 and SAMR1 with WD. In SAMR1, WD did not modify Phe contraction in spite of inducing major changes in the mechanisms of regulation of contractile responses. Although WD increased NAD(P)H-oxidase-derived O_2^- and augmented peroxynitrite formation, we found an increase of inducible NOS (iNOS)-derived NO production which may contribute to maintain Phe contraction in SAMR1 WD. On SAMP8, WD significantly decreased Phe-induced contractions when compared with SAMP8 under normal chow. This response was not dependent on changes of NOS expression, but rather as consequence of increased antioxidant capacity by superoxide dismutase (SOD1). A similar constrictor influence from cyclooxygenase (COX) pathway on Phe responses was found in SAMR1 and SAMP8 ND. However, WD removed that influence on SAMR1, and produced a switch in the balance from a vasoconstrictor to a vasodilator component in SAMP8. These results were associated to the increased COX-2 expression, suggesting that a COX-2-derived vasodilator prostaglandin may contribute to the vascular adaptations after WD intake. Taken together, our data suggest that WD plays a detrimental role in the vasculature of non-senescent mice by increasing pro-inflammatory (iNOS) and pro-oxidative signaling pathways and may contribute to increase vascular senescence. In senescent vessels, however, WD triggers different intrinsic compensatory alterations which include increase of antioxidant activity by SOD1 and vasodilator prostaglandin production *via* COX-2.

1. Introduction

With the increasing life expectancy of the world population, vascular senescence is becoming a major independent risk factor for cardiovascular disease (CVD) (Lakatta, 2003; Lakatta and Levy, 2003). Both epidemiological and observational studies have described functional and structural alterations in the vasculature with advanced age that share similarities to those observed in early states of CVD development (Lakatta, 2003; Lakatta and Levy, 2003). With aging the vascular wall of large elastic arteries gradually stiffs (Redheuil et al., 2010) and endothelial-dependent regulation of vascular tone is impaired (Herrera et al., 2010). Those changes in the large arterial systems are now fully recognized to play a critical role in the progression of CVD, as they are positively associated with systolic hypertension, coronary heart disease, stroke and heart failure (Lakatta, 2003; Lakatta and Levy, 2003).

In general terms vascular aging could be described as a simple consequence of mechanical fatigue and oxidative stress (de Grey, 2006; O'Rourke and Hashimoto, 2007). In both conditions a number of physiological and morphological changes take place, which alter cardiovascular function and lead to subsequently increased risk of CVD, even in healthy asymptomatic individuals (Redheuil et al., 2010). However vascular aging is a more complex process involving a myriad of signaling pathways which can be delayed or accelerated. In fact, a growing number of studies (including ours) have shown that environmental factors can slow down or accelerate this set point (Jimenez-Altayo et al., 2012; Nilsson, 2008). Under those influences vascular aging process seems to take a more rapid course, eventually resulting in premature CVD manifestations (Nilsson, 2008). Among those environmental factors, food intake and type of diet have been identified as determining factors in the aging process. Emerging evidences have proposed that calorie restriction can slow down aging and prevent aging-associated disease (Lane et al., 2000; Shinmura et al., 2011; Ungvari et al., 2010). However little is known about how diet contents can modulate vascular senescence and functioning. Seeing that the consumption of high-fat diet has been considerably grown in western countries – therefore the

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name Western-type diet (WD) – it is crucial to increase our understanding of how this diet regimen affects vascular senescence and function.

The aging-associated vascular pathophysiology has been extensively studied in mice models because their physiological and genetic parallel with human cardiovascular system (Yutzey and Robbins, 2007). In these studies we have used a murine model of accelerated senescence (senescence accelerated mice — SAM), which has been established by our group and others as suitable model to study the pathophysiology of aging in a convenient and standard time course. More specifically we used the SAM prone strain (SAMP8) which has shown signs of accelerated senescence of the cardiovascular system compared with the senescence-accelerated resistant strain (SAMR1) (Llorens et al., 2007; Novella et al., 2010, 2011). We have found increased contractility and endothelial dysfunction in aortas from 6- to 7-months-old SAMP8 compared with SAMR1 (Novella et al., 2010, 2011). Previous observations on long-term feeding with high-fat diet, reveled the presence of a more prevalent and extensive fatty lesions and macrophage invasion in aortas of SAMP8 than SAMR1 mice, suggesting that senescence may increase atherogenicity (Fenton et al., 2004). In these studies we aimed to determine whether and how Western-type diet affects vascular function in non-senescent and senescent mice, to establish a potential correlation between this food regimen with the progression of vascular senescence and senescence-associated changes of vascular reactivity in mice aorta.

2. Methods

2.1. Animals and diet

Female senescence-accelerated resistant and senescence-accelerated prone mice were obtained from the breeding stock at Parc Cientific de Barcelona and housed at Animal Facility of University of Barcelona according to institutional guidelines (constant room temperature 22 °C, 12 h light/dark cycle, 60% humidity, and water *ad libitum*). All protocols were approved by the Institutional Ethics Committee at the University of Barcelona (Comitè Ètic d'Experimentació Animal — CEEA protocol: 625/08), conformed to the National Institute of Health Guide for the Care and Use of Laboratory Animals. Both SAMR1 (n = 48) and SAMP8 (n = 47) were randomly separated at 5 months of age into 2 groups: 1) standard mice chow (normal diet, ND) [Harlan Teklad mouse breeding and maintenance diet, TRM.9607] or 2) WD [Harlan Teklad Western adjusted calorie diet, TD.88137]. Both diets were given *ad libitum* for 8 weeks following the protocol previously described (Jimenez-Altayo et al., 2012). Mice age and diet regimen were chosen based on preliminary studies to determine the beginning of vascular senescence in SAMP8, but not in SAMR1 (Jimenez-Altayo et al., 2012; Novella et al., 2010). The abdominal aorta was dissected and kept in ice-cold physiological salt solution and prepared for different experiments essentially as described (Martinez-Revelles et al., 2012).

2.2. Determination of senescence in thoracic aorta

Thoracic aortas were fixed in 4% paraformaldehyde for 24 h, rinsed with phosphate-buffered saline (PBS) repeatedly, and subjected to β-galactosidase (β-gal) staining overnight using a commercial kit (Roche Applied Sciences). The stained abdominal aortas were washed with PBS and then photographed with a Panasonic digital camera. The percentage of the β-gal staining area in comparison with the entire thoracic aorta area was quantified using the ImageJ Software.

2.3. Vascular reactivity

Intact segments (2 mm) of dissected thoracic aorta were set up on an isometric wire myograph (model 410 A; J.P. Trading, Aarhus, Denmark) as described (Martinez-Revelles et al., 2012). After 30 min equilibration, contractile responses to Phe (10^{-9} to 10^{-5} M) were performed in the absence or presence of the nonselective nitric-oxide synthase (NOS) inhibitor Nω-nitro-L-arginine methyl ester (L-NAME; 10^{-4} M); the O_2^- scavenger tempol (10^{-3} M), or the nonselective cyclooxygenase (COX) inhibitor indomethacin (10^{-5} M). Each treatment was added 30 min before and kept throughout the experiments.

2.4. Measurement of O_2^- production and NAD(P)H oxidase activity

Superoxide anion (O_2^-) production was determined *in situ* in aortic sections by dihydroethidium (DHE), following the methodology described previously with few modifications (Jimenez-Altayo et al., 2007; Martinez-Revelles et al., 2008). Aortic sections (4 μm) were incubated at 37 °C for 30 min with 5 μM DHE in Hank's Balanced Salt Solution. Following incubation period, DHE was removed and coverslips were mounted on slides using ProLong Gold antifade reagent with DAPI (Invitrogen). Parallel sections were incubated with polyethylene glycol SOD (PEG-SOD; 500 U/ml); L-NAME (300 μM) and Apocynin (100 μM) to evaluate the specificity of the signal and potential sources of superoxide. Sections were visualized through a fluorescent microscope (Olympus SX-31, ×40) with a 10× and 40× objective lens (Olympus). For each image, light was passed through a different excitation filter: 1) 350 nm (for DAPI); 2) 490 nm (for autofluorescence); and 3) 590 nm (for ethidium bromide, EB). At least three aortic sections were recorded from each animal. Images were merged using Mac Biophotonic ImageJ Software and EB fluorescence was expressed as percentage of fluorescence elicited by DAPI. Lucigenin-enhanced chemiluminescence was used to determine O_2^- production after adding excess NADPH (100 μM), the substrate for NAD(P)H oxidase, essentially as described (Novensa et al., 2011).

2.5. Measurement of hydrogen peroxide production

Hydrogen peroxide formation was determined along vascular wall by the ferric-xylenol orange hydroperoxide assay, adapted from a methodology previously described (Dantas et al., 2002; Jimenez-Altayo et al., 2012). Aortic sections were initially incubated with 10% methanol (v/v) for 20–30 min at room temperature, followed by incubation of reaction mixture containing 25 mM ammonium ferrous (II) sulfate; 2.5 M H_2SO_4; 4 mM butyl-hydroxytoluene and 125 μM xylenol orange in methanol. Colored images were captured with a microscope (Olympus SX-31, ×40) using Soft Cell software. Quantitative analysis of hydrogen peroxide production was performed with ImageJ software. Percentage of labeled area was measured in at least two rings of each animal.

2.6. Western blot analysis

Equal amount of protein from each sample (25 μg) was resolved by SDS-PAGE on 4–12% gels and electroblotted onto nitrocellulose. Membranes were incubated overnight at 4 °C in phosphate-buffered saline with 0.1% (v/v) Tween 20 (PBST) containing 5% milk and specified primary antibodies as follows: monoclonal mouse anti-eNOS, 1:1000 (BD Transduction Laboratories); polyclonal rabbit anti-iNOS, 1:500 (Calbiochem); polyclonal rabbit anti-nitrotyrosine 1:500 (Merk Millipore); 1:500 polyclonal goat anti-gp91^{phox} (Santa Cruz Biotech); 1:500 polyclonal rabbit anti-p47^{phox} (Santa Cruz Biotech); 1:500 monoclonal mouse anti-p22^{phox} (Santa Cruz Biotech); 1:500 monoclonal rabbit anti-SOD1 (Santa Cruz Biotech). After incubation with specific with horseradish peroxidase-labeled specific secondary antibodies in PBST containing 1% milk and additional washes, chemiluminescent signal was visualized by LAS4000 imaging system (GE Healthcare). Densitometric analyses of Western blots were performed using a Mac Biophotonic ImageJ Software. All membranes were reblotted using a monoclonal antibody anti GAPDH (1:2500; Santa Cruz Biotechnology) as a loading control. Data were normalized to corresponding values of GAPDH densitometry.