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Western diet consumption promotes vascular remodeling in non-senescent mice consistent with accelerated senescence, but does not modify vascular morphology in senescent ones

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ABSTRACT

Senescence accelerated mice (SAM) are susceptible to developing vascular dysfunction and remodeling. Food intake and type of diet have also been identified as determining factors in vascular remodeling. However, the interplay between senescence and diet in vascular remodeling is largely unknown. We aimed to analyze structure of large (aorta) and small (mesenteric; MA) arteries from seven-month-old SAM prone (SAMP8) and resistant (SAMR1) mice that received a Western-type high-fat diet (WD; 8 weeks). Aortic structure was assessed by morphometric analysis of hematoxylin and eosin-stained cross sections, and collagen content by qRT-PCR, immunofluorescence and picrosirius red. In MAs, structural and mechanical properties were measured by pressure myography; elastin and collagen content by qRT-PCR and immunofluorescence; nuclei distribution by confocal microscopy; and apoptosis by qRT-PCR and TUNEL assay. In aorta, wall thickness (WT), but not cross-sectional area (CSA), was increased by senescence, and WD only increased WT in SAMR1. WD intake, but not senescence, was associated with increased collagen deposition. In MAs, senescence diminished WT and CSA, without altering collagen and elastin deposition, reduced the number of MA wall cells, and increased pro apoptotic activation. WD consumption promoted in SAMR1 the same remodeling observed with senescence, while in SAMP8 the senescence-associated changes remained unaffected. The mechanisms involved in WD-induced MA remodeling in SAMR1 mimicked those observed in senescence per se. Our study reveals qualitatively different remodeling in aortas and MAs from senescent mice. Consumption of a WD induced remodeling of the SAMR1 vasculature similar to that induced by senescence, while it did not promote any further alteration in the latter. Therefore, we propose that increased consumption of fat-enriched diets could promote accelerated senescence of the non-senescent vasculature, although it does not exacerbate vascular remodeling during senescence.

1. Introduction

Arterial wall remodeling is considered an early indicator of cardiovascular complications. Along with the well-known impairment of endothelial function (El Assar et al., 2012; Herrera et al., 2010), it is increasingly accepted that aging progressively modifies structural and mechanical properties of large and small arteries contributing to the progression of vascular pathologies (Briones et al., 2007; Fornieri et al., 1992; Hajdu et al., 1990; Hausman et al., 2012; Moreau et al., 1998; Redheuil et al., 2010; Wang and Lakatta, 2002). Each cardiac cycle per se induces a mechanical constraint on the arteries, leading to tissue fatigue. In this scenario, the sequence of events leading to vascular remodeling with aging is not completely understood, but it is proposed that age-related, naturally occurring stiffening of large arteries may induce reactive changes in microvascular structure and function, compromising resting blood flow and contributing to end-organ damage (Mitchell, 2008; Moreau et al., 1998). Changes in arterial wall integrity can be orchestrated by modifications of their cellular and/or extracellular components. Alterations in cell number or morphology and changes in extracellular matrix composition have been documented. Thus, the mechanisms underlying remodeling of large and small arteries with aging have been partly associated with alterations of smooth muscle cells, collagen and elastin (Briones et al., 2007; Cliff, 1970; Dao et al., 2005; Fornieri et al., 1992; Wang et al., 2010).

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Besides aging, weight gain is proposed as an independent cardiovascular risk factor and has been closely associated with changes in vascular morphology (Deutsch et al., 2009; Eschert et al., 2009; Ma et al., 2010; Tavakoli et al., 2011). Recent evidence has shown that food intake and type of diet are determining factors in the aging process (Nilsson, 2008). In this sense, studies have proposed that obesity may activate pathways consistent with accelerated vascular aging (Barton, 2010). The life expectancy of the population and exposure to high-fat diets are increasing worldwide. Remarkably, few studies to date have addressed the interaction of mechanisms through which aging and diet-induced obesity promote the development of cardiovascular disease. Therefore, analysis of the potential modifications of vascular structure induced by the interaction between diet-induced weight gain and aging is clearly warranted.

Most studies are performed in advanced stages of several cardiovascular pathologies, which may not provide insights on the mechanisms associated with their initiation and may interfere with promoting effective interventions. The senescence accelerated mouse (SAM) is a suitable model to study cardiovascular physiological changes during aging (Jiménez-Altayó et al., 2013; Llorens et al., 2007; Novella et al., 2010, 2013a,b; Onetti et al., 2013; Reed et al., 2011; Zhu et al., 2001). These animals age fast and predictably, without vascular damage at an early age (Butterfield and Poon, 2005; Novella et al., 2013a,b). Mesenteric arteries (MAs) from SAMP8 at an early stage of vascular senescence show vascular adaptation and are susceptible to functional changes in response to high-fat intake (Jiménez-Altayó et al., 2013). In aortas from SAMP8, high-fat intake induces senescence and triggers different molecular compensatory mechanisms that cause functional changes (Onetti et al., 2013). The observed functional modifications of the SAM vasculature call our attention to the possible changes in structure, as altered vascular function is tightly related to the development of remodeling (Heagerty et al., 2010). In the present study, we hypothesized that administration of a high-fat diet, also known as Western diet (WD), could lead to vascular remodeling similar to that promoted by senescence and worsen the damage exerted by senescence. For this purpose, we analyzed structure of large (aorta) and small (mesenteric) arteries from SAM at an early stage of vascular senescence following WD administration, and examined the mechanisms involved.

2. Methods

2.1. Animals and diet

Female SAMR1 and SAMP8 mice were obtained from the breeding stock at Parc Científic de Barcelona, which began with matrices from Harlan (Harlan Laboratories UK, Bicester, UK) housed according to institutional guidelines (constant room temperature at 22 °C, 12 h light/dark cycle, 60% humidity, and water ad libitum). Experiments were approved by the Ethics Committee of the Universitat de Barcelona and conformed to the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. Previous studies of our group demonstrated that at 6–7 months of age SAMP8 but not SAMR1 manifest vascular senescence (Jiménez-Altayó et al., 2013; Novella et al., 2010, 2013a,b; Onetti et al., 2013). Both SAMR1 ($n = 44$) and SAMP8 ($n = 39$) were randomly separated at 5 months of age into 2 groups receiving ad libitum for 8 weeks: 1) a standard mice chow (normal diet; ND; Harlan Teklad mouse breeding and maintenance diet, Teklad Global Diet-2018), containing 4.3% fat by weight (0.0023% cholesterol); and 2) a WD (Harlan Teklad Western Adjusted Calories Diet, TD.88137) (Fenton et al., 2004; Jiménez-Altayó et al., 2013; Onetti et al., 2013), containing 21.2% fat by weight (0.15% cholesterol). Both diets were given as pellets following the protocol previously described (Jiménez-Altayó et al., 2013). Blood glucose was determined by tail prick at a regular time-point using a strip-operated reflectance meter (Accu-Check Aviva, Roche Diagnostics, Basel, Switzerland). At 7 months old, all mice were anesthetized with sodium pentobarbitone (40 mg/kg; i.p.) and decapitated. The thoracic aorta and the mesenteric arcade were placed in ice-cold physiological salt solution (PSS; composition in mM: NaCl 112.0; KCl 4.7; $CaCl_2$ 2.5; KH_2PO_4 1.1; $MgSO_4$ 1.2; $NaHCO_3$ 25.0 and glucose 11.1) gassed with 95% O_2 and 5% CO_2.

2.2. Tissue preparation

Segments of thoracic aorta [morphometry, qRT-PCR, picrosirius red and immunofluorescence] and first-order branches of the superior MA [pressure myography, qRT-PCR, nuclei distribution, immunofluorescence and TUNEL assay] were dissected free of fat and connective tissue in ice-cold PSS. The vessels were prepared essentially as previously described (Jiménez-Altayó et al., 2013; Onetti et al., 2013).

2.3. Aortic morphometry

Since the dissection and fixation procedures considerably affect measurements of the aortic wall thickness and lumen diameter, aortas were carefully perfused in situ with phosphate-buffered saline (PBS) at room temperature through a needle positioned into the left ventricle. The perfusion pump speed was set at 12 ml/min, which corresponds to the cardiac output of anesthetized mice (Sarin et al, 1990). The outlet was created by cutting the lower vena cava. Following 10 min of PBS perfusion, the buffer was replaced with 4% paraformaldehyde (PFA), and the perfusion/fixation was continued for further 10 min. Finally, aortas were carefully dissected and cleaned of fat and connective tissue, post-fixed overnight in 4% PFA and embedded in Tissue-Tek OCT embedding medium (Sakura Finetek Europe, Zoeterwoude, The Netherlands). Morphometric determination of aortic lumen, wall thickness (WT) and cross-sectional area (CSA) was performed using hematoxylin and eosin staining and analyzed using ImageJ (NIH Image). The perimeter of the vessel lumen and outer wall was taken as the circumference (C) of a circle. The lumen (D_i) and external (D_e) diameters were determined from the equation $D = C/\pi$ (assuming that the vessel cross-sections are circular in vivo). Six-eight serial cross-sections were measured and averaged from each vessel segment. The aortic WT was calculated as $WT = (D_e - D_i)/2$ and CSA was $CSA = (\pi/4) \times (D_e^2 - D_i^2)$.

2.4. Aortic smooth muscle cell culture and growth

Smooth muscle cells (SMC) were isolated from 7 month-old SAMR1 and SAMP8 mice aortas by enzymatic digestion and cultured as reported (Cidad et al, 2010). For proliferation, SMC were sparsely plated (2.5×10^4 cells/cm^2) in 6-well dishes with DMEM-F12 media supplemented with 10% fetal bovine serum (FBS) and 1% ITS media supplement (Sigma-Aldrich, St Louis, MO, USA). After 7 days cell number was determined with an automated hemocytometer (Countess® Automated Cell Counter, Life Technologies, Thermo Fisher Scientific Inc., Waltham, MA, USA). Cell proliferation was expressed as the ratio between counted and seeded cells.

2.5. Pressure myography

Structural, mechanical and myogenic properties of the MA were studied with a pressure myograph (Danish Myo Tech, model P100; J.P. Trading, Aarhus, Denmark), as previously described (Caracuel et al., 2012; Jiménez-Altayó et al., 2009). Briefly, vessels were placed on two glass microcannulas and carefully adjusted so that the vessel walls were parallel without stretching. Intraluminal pressure was then raised to 140 mm Hg, and the artery was unbuckled by adjusting the cannulas. Afterward, the artery was left to equilibrate for 1 h at 70 mm Hg in gassed PSS (37 °C). Intraluminal pressure was reduced to 3 mm Hg, and a pressure–diameter curve (3–120 mm Hg) was obtained. Internal and external diameters (D_{iCa} and D_{eCa}) were measured for 3 min at each intraluminal pressure. The artery was left to equilibrate for 30 min at