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Arterial stiffness is associated with adipokine dysregulation in non-hypertensive obese mice

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ABSTRACT

The aim of this study was to characterize alterations in vascular structure and mechanics in murine mesenteric arteries from obese non-hypertensive mice, as well as their relationship with adipokines. Four-week old C57BL/6J male mice were assigned either to a control (C, 10% kcal from fat) or a high-fat diet (HFD, 45% kcal from fat) for 32 weeks. HFD animals weighed 30% more than controls ($p < 0.001$), exhibited similar blood pressure, increased leptin, insulin and superoxide anion ($O_2^{\bullet-}$) levels, and reduced adiponectin levels and nitric oxide (NO) bioavailability. Arterial structure showed an outward remodeling with an increase in total number of both adventitial and smooth muscle cells in HFD. Moreover, HFD mice exhibited an increased arterial stiffness assessed by β-values (C = 2.4 ± 0.5 vs HFD = 5.3 ± 0.8; $p < 0.05$) and aortic pulse wave velocity (PWV, C = 3.4 ± 0.1 vs HFD = 3.9 ± 0.1; $p < 0.05$). β-Values and PWV positively correlated with leptin, insulin or $O_2^{\bullet-}$ levels, whereas they negatively correlated with adiponectin levels and NO bioavailability ($p < 0.01$). A reduction in fenestrae number together with an increase in type-I collagen amount ($p < 0.05$) were observed in HFD. These data demonstrate that HFD accounts for the development of vascular remodeling and arterial stiffness associated with adipokine dysregulation and oxidative stress, independently of hypertension development.

1. Introduction

Obesity is associated with progressive vascular dysfunction leading to elevated morbidity and mortality due to early cardiovascular events [1]. Mechanisms of vascular dysfunction include vascular remodeling and arterial stiffness, both actively contributing to the development of cardiovascular disease [2–4].

Obesity has emerged as a potential risk factor for arterial remodeling in both humans and rats [2–5]. In this direction, severe human obesity has been associated with profound structural alterations of subcutaneous small resistance arteries [6]. Likewise, studies performed in obese Sprague Dawley (SD) [7], obese Zucker [8] and diabetic rats [3] show vascular remodeling of middle cerebral and/or mesenteric arteries. Nevertheless, in most cases, the concomitant presence of diabetes and hypertension [9], both linked to vascular remodeling, makes difficult to discriminate the role of obesity per se in the development of the observed structural abnormalities.

Chronic alterations in vascular structure may lead to significant changes in mechanical properties, such as compliance and distensibility [3], thus accounting for arterial stiffness, an independent risk factor for cardiovascular disease [10]. Obesity is associated with an increase in aortic pulse wave velocity (PWV) and/or intrinsic stiffness (assessed by the stress–strain relationship) in human subcutaneous small resistance arteries [6,11–13], as well as in aorta of high-fat/high-sucrose SD [7] or in genetic models of obesity, i.e., *ob/ob* mice [14] and insulin-resistant Zucker fa/fa rats [8]. Recent evidence suggests that arterial stiffness associated to obesity might appear in the absence or prior to the development of hypertension in patients with metabolic syndrome [13]. In obese children, arterial stiffness seems to be influenced by body mass index and pulse pressure independently of systolic and diastolic blood pressure values [15]. Conversely, weight loss in overweight and obese individuals is associated with a reduction in arterial stiffness [7].

Abbreviations: AIx, augmentation index; C, control; CSA, cross-sectional area; DAF-2DA, 4,5-diaminofluorescein diacetate; DHE, dihidroethidium; DIO, diet-induced obesity; EEL, external elastic laminae; eNOS, endothelial nitric oxide synthase; HFD, high-fat diet; IEL, internal elastic laminae; KH, Krebs–Henseleit solution; MA, mesenteric artery; NO, nitric oxide; NOX, NADPH oxidase activity; $O_2^{\bullet-}$, superoxide anion; PFA, paraformaldehyde; PWV, pulse wave velocity; SMC, smooth muscle cells.

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In this context, some studies suggest a possible link between adipokine levels and the development of arterial stiffness in patients with abdominal obesity [16] or increased adiposity [17].

Passive arterial mechanical properties are mainly conferred by collagen, elastin content and elastin organization [18,19]. Enhanced vascular stiffness of resistance arteries has been attributed to increases in collagen content [20,21], non-fibrous extracellular matrix proteins, and adhesion molecules [for review, see Ref. [22]], as well as to alterations in elastic fiber organization in the internal elastic lamina [23]. According to this, several studies have shown a link between arterial stiffness and abnormal increase in the collagen/elastin ratio in hypertension [18,24–26]. However, very few studies [11,12] have been performed in the context of obesity, and the role of obesity per se in the development of mechanical abnormalities remains to be elucidated.

In this context, the aim of this study was to prove the hypothesis of a direct link between obesity-derived adipokine dysregulation [27,28], vascular remodeling and arterial stiffness in obesity, without the influence of hypertension as confounding factor. Therefore, we sought to characterize structural and mechanical changes in a mouse model of long-term diet-induced obesity (DIO), which exhibits endothelial dysfunction together with an increase of oxidative stress, but does not develop hypertension [29]. We have analyzed in mesenteric arteries: i) vascular structure, ii) mechanical properties, iii) elastin content and organization, iv) types I and III collagen contents, iv) the correlation between adipokine dysregulation and arterial stiffness and v) the correlation between oxidative stress and arterial stiffness.

2. Material and methods

2.1. Animals and dietary treatment

Four-week old male C57BL/6J mice (Harlan, Spain) weighing 16–18 g were housed under controlled light (12-hour light/dark cycles from 8:00 am to 8:00 pm) and temperature (22–24 °C) conditions with standard food and water ad libitum. After one week, animals with similar average body weight, were divided into two groups and housed 8–10 per cage and assigned either to a control (C) or to a high-fat diet (HFD). Control (D12450B, 10 kcal% fat, 70 kcal% carbohydrates and 20 kcal% protein; 3.85 kcal/g) and high-fat (D12451, 45 kcal% fat, 35 kcal% carbohydrates, 20 kcal% protein; 4.73 kcal/g) diets were supplied by Test Diet Limited BCM IPS Ltd. (London, UK). HFD and their respective control mice had free access to food during 32 ± 1 weeks. The investigation conform the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes and it was approved by the ethics committee of the University CEU-San Pablo (SAF 2009-09714, SAF2011-25303).

2.2. Pulse wave velocity and arterial wave reflection index determination

On the last day, both carotid and femoral arteries were catheterized under anesthesia (80 $mg \cdot kg^{-1}$ ketamine hydrochloride and 12 $mg \cdot kg^{-1}$ xylazine hydrochloride, ip) and blood pressure waves were recorded in a PowerLab system (ADInstruments). Pulse wave velocity (PWV) represents the pressure waveform that travels along the aorta and large arteries during each cardiac cycle and it was calculated with the following formulae: D (meters) / Δt (seconds), where the time delay (Δt) was measured by using the two pressure waves (carotid and femoral) and D was the distance between the two arteries. Arterial wave reflection was determined by using arterial pressure waveforms from the right carotid artery and the augmentation index (AIx, magnitude of wave reflection) was calculated as previously described [30]. After pulse wave determination, anesthetized animals were euthanized by decapitation. The mesenteric bed was immediately dissected, blood was collected in chilled EDTA-coated polypropylene tubes and plasma samples were frozen at −80 °C for further analysis.

2.3. Plasma measurements

Plasma leptin and adiponectin concentrations were analyzed by specific RIA for murine leptin (Linco Research) and adiponectin (Linco Research). Insulin was determined by means of a specific EIA kit for mouse insulin (Mercodia).

2.4. Structural and mechanical properties in mesenteric arteries

Mesenteric bed was removed and placed in Krebs–Henseleit solution (KH, in mM: 115 NaCl, 4.6 KCl, 2.5 $CaCl_2$, 25 $NaHCO_3$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 0.01 EDTA and 11.1 glucose). A first-order branch of mesenteric artery (MA) was isolated from the mesenteric bed and was carefully cleaned of surrounding adipose tissue under a dissecting microscope. MAs structural and mechanical properties were studied with a pressure myograph (Model P100, Danish Myo-Tech), as previously described [23,31]. Briefly, vessels were placed on two glass cannulas, secured with surgical nylon suture and vessel length was adjusted so that the vessel walls were parallel without stretch. To equilibrate MA segments, intraluminal pressure was set at 70 mm Hg for 60 min at 37 °C in calcium-free KH ($0Ca^{2+}$; omitting calcium and adding 10 mM EGTA), bubbled with carbogen (95% O_2/5% CO_2). Thereafter, intraluminal pressure was increased at 20 mm Hg intervals (3, 20, 40, 60, 80, 100, 120, and 140 mm Hg), and external and internal diameters (D_{i0Ca}, D_{e0Ca}) were recorded at each pressure level with a video camera coupled to Myoview software. After maximal relaxation in $0Ca^{2+}$, MA segments were pressure-fixed at 70 mm Hg with 4% paraformaldehyde (PFA, in 0.2 mol/l phosphate buffer, pH 7.2–7.4) at 37 °C for 45 min and stored at 4 °C for confocal microscopy studies.

From the D_{e0Ca} and D_{i0Ca} values we calculated structural [wall thickness, cross-sectional area (CSA), and wall-to lumen ratio] and mechanical parameters [incremental distensibility, circumferential wall strain, circumferential wall stress, and β-values obtained from stress–strain relationship] as described [23].

2.5. Confocal microscopy

2.5.1. Confocal microscopy study of nuclei distribution

Pressure-fixed intact MA arteries were stained with the nuclear dye DAPI (1:500, Molecular Probes) for 15 min at room temperature (RT) in the darkness. After washing, the arteries were mounted on a slide provided with a small well of spacers to avoid artery deformation, filled with Citifluor (glycerol-antifade agent; Sigma Aldrich) mounting medium, and visualized with a Leica TCS SP5 confocal system (Leica Microsystems) and the cell nuclei in the adventitia, media, and endothelium were visualized at excitation 405 nm/emission 410–475 nm. For each artery, a single image was captured in the midpoint of the artery with a ×20 objective. In addition, three randomly selected regions were visualized with a ×63 objective zoom 4. In each of the regions, stacks of 1-μm-thick serial optical sections were taken from the first visible adventitial cell nuclei to the first visible endothelial cell nuclei. An additional group of images focusing of the endothelial monolayer were captured along the entire segment length. Metamorph images analysis software (Universal Imaging) was used for quantification. To allow comparison of C and HFD animals, the following calculations were performed on the basic of 1-mm-long segments: artery volume (in mm^3) (volume = wall CSA (mm^2) × 1 mm); total number of adventitial and smooth muscle cells (cell n = n of nuclei per stack × n of stack per artery volume). Endothelial cells were quantified in several single images obtained along the arterial length and calculated per area, since endothelium is a monolayer. Data are expressed as endothelial cell number per luminal surface area of each vessel, which was calculated from the internal diameter measured from images captured with the ×20 objective. CSA (μm^2) was calculated on the basis of the wall and lumen measurements.

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