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Loss of Compliance in Small Arteries, but Not in Conduit Arteries, After 6 Weeks Exposure to High Fat Diet

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Abstract Arterial stiffness is a key marker in metabolic diseases and can be evaluated by arterial compliance. Most compliance measurements are performed in large conduit arteries in advanced stage of metabolic diseases, which may not provide information on mechanisms associated with the initiation of the pathology. For this reason, we compared arterial compliance of two different size arteries: carotid and a smaller artery (thoracodorsal artery, TDA). The arterial compliance was compared between control and high fat-fed mice for 6 weeks. We show that the compliance of the TDAs was dramatically reduced in high fat-fed mice whereas the compliance of the carotids remained unchanged. An abundance of collagen deposition in the media/adventitia of the carotids and TDAs was observed in high fat-fed mice. These results demonstrate that the structural and mechanical properties of small arteries are rapidly altered even after only 6 weeks of high fat feeding.

Keywords Compliance · Pressure–diameter response · Obesity · Metabolic diseases

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Introduction

Obesity affects 30% to 60% of the population in the USA and their cardiovascular complications are a leading cause of morbidity, mortality, and economic burden [1]. Furthermore, there is a rise in the prevalence of obesity for the next decades due to a caloric-enriched diet: the trends project 65 million more obese adults in the USA by 2030 [1]. Caloric-enriched diet not only leads to obesity but also leads to associated risk factors including type 2 diabetes, hypercholesterolemia, and hyperlipidemia, regrouped under the term "metabolic disease," which increases the risk of cardiovascular diseases [2].

An early indicator of cardiovascular complications due to metabolic disease is arterial stiffening, which occurs due to arterial wall remodeling [3, 4]. This arterial remodeling involves the reorganization of existing cells, such as changes in vascular smooth muscle cell growth and migration and changes in extracellular matrix (ECM) composition [5–8]. The ECM is thus an important determinant of the arterial wall integrity as well as of the arterial compliance, which is defined by the change in lumen area for a given change in intraluminal pressure. Although it is well accepted that arterial compliance of the carotids is altered and that there is a strong arterial remodeling in metabolic diseases, most of these studies have been performed on animal models with advanced stage metabolic diseases (e.g., high fat diet for more than 10 weeks). Therefore, there remains a need to investigate an eventual premature arterial remodeling in early stages of metabolic diseases.

Alteration in arterial compliance is a key marker for arterial stiffness and it is commonly measured in pathological conditions in large conduit arteries (e.g., carotids) using pulse

wave velocity, which corresponds to the speed at which an arterial waveform travels between two points along a vessel [9]. Although this method is noninvasive, it can be inaccurate when performed in small animals since the recording points along the vessel are closer together. For the same reason, this method cannot be used on smaller arteries (neither in humans nor in animals). Because of this, there are few reports on mechanical properties of small arteries. Also, previous studies have reported that structural alterations of small arterial walls are the most potent predictor of cardiovascular diseases, indicating a strong need to study these arteries in pathological conditions [10].

The aim of the present study was to examine the compliance of two arteries: a large conduit artery, the carotid and a smaller artery, the thoracodorsal artery (TDA) both isolated from 6-week high fat-fed mice and to compare their compliance to control conditions. We further correlated the compliance results with histological alterations observed using several histological stains and transmission electron microscopy. Our results indicate that after 6 weeks of high fat diet, several histological changes were observed and there was a dramatic alteration of the compliance of small arteries, but the compliance of the larger conduit arteries remained unchanged. To our knowledge, this is the first study of arterial stiffness of small arteries in a mouse model of early stage of obesity.

Material and Methods

Animals

Male mice (8–10 weeks; C57Bl/6) were purchased from Taconic and used according to the *University of Virginia Animal Care and Use Committee guidelines*. Mice were separated in two groups: a control group, fed with a normal diet (3.1 kcal/g, 0% cholesterol, 5.8% fat, Harlan Laboratories) and a second group where mice were fed with a caloric-rich diet (5.45 kcal/g, 0.2% cholesterol, 35.5% fat; Bio-Serv) for 6 weeks.

Compliance Measurements

Mice were sacrificed using CO_2 asphyxia; the TDA and the carotid were isolated and placed in Krebs-HEPES containing (in mM) NaCl 118.4, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 4, KH_2PO_4 1.2, $CaCl_2$ 2, Hepes 10, glucose 6, and supplemented with 1% BSA. Arteries were free of surrounding tissue and were placed in an arteriograph (Danish MyoTechnology, DMT, Ann Harbor, MI) where they were cannulated at both ends with glass micropipettes and secured with 10–0 nylon monofilament suture. Arteries were perfused with Krebs-HEPES supplemented with 1% BSA and superfused with a calcium free Krebs-HEPES containing ethylenbis-(oxyethylenenitrolo) tetra-acetic acid (EGTA, 2 mmol/L) and sodium nitroprussiate (10 μmol/L). For experiments performed on TDA, the arteriograph was placed on an Olympus IX-71 microscope; the TDAs were visualized with a 20× objective and were subjected to a gradient of pressure from 10 to 140 mmHg with a 5-min stabilization period for each pressure to measure the passive diameter using the Slidebook software. For experiments on the carotids, the arteriograph was placed on a DMT microscope and the carotids were monitored with a 10× objective. The intraluminal pressure was increased from 10 to 170 mmHg by steps of 20 mmHg and the lumen diameter was measured in μm using the DMT vessel acquisition suite. The lumen area was calculated in μm^2, for each pressure step, using the lumen diameter values according to the formula: lumen area$=\pi\times$lumen diameter$^2/4$. The compliance was measured in μm^2/mmHg using the formula: compliance$=\Delta$ lumen area/Δ pressure in which Δ lumen area is the change in lumen area induced by a change of intraluminal pressure (Δ pressure).

Histology

Mice were sacrificed using CO_2 asphyxia and were transcardially perfused with free calcium Krebs-HEPES supplemented with heparin (10 U/ml) and then perfused with 4% paraformaldehyde (PFA). The carotids and TDA were isolated, free of surrounding tissues and placed in 4% PFA for 1 h at 4°C and in ethanol 70% for further paraffin embedding. Paraffin blocks were further cut in 4 to 5-μm-thick cross sections, which were further subjected to different stains: hematoxylin and eosin (H&E), periodic acid-Shiff (PAS), Gömöri trichrome (G-Tri), and Masson's trichrome (M-Tri). Sections were further observed using a 40× objective mounted on a Nikon Microphot FXA microscope and captured with the Image Pro Software.

Transmission Electron Microscopy

Mice were sacrificed using CO_2 asphyxia and were transcardially perfused with free calcium Krebs-HEPES-heparin as described above and then perfused with 4% PFA supplemented with 2.5% glutaraldehyde. The carotids and the TDA were isolated, placed in 4% PFA+2.5 % glutaraldehyde at 4°C until fixation in 1% osmium tetraoxide followed by dehydration in a gradient of alcohol and embedding in Epon. Ultrathin sections (75 nm) were cut, carbon coated and imaged with a JEOL 1230 as previously described [11].

Data Analysis

Passive diameter and compliance are expressed in mean±SEM and data from control and high fat-fed groups were compared with a Student's *t* test using Origin software or a two-way

ANOVA using the GraphPad software. $P<0.05$ was considered significant.

Results

Compliance of Carotid and TDA in High Fat-Fed Mice

Mice fed a high fat diet for 6 weeks had a significantly higher body weight as compared to the control group (34.8±1.99 and 24.1±0.72 g, respectively). The passive diameter was measured at increasing intraluminal pressures on carotids and TDAs isolated from control and high fat-fed mice. The passive diameter of control TDAs pressurized at 10 mmHg was 167±8.25 μm and rapidly increased to the maximal passive diameter, approximately 260 μm (Fig. 1a). The passive diameter of TDAs isolated from high fat-fed mice was significantly higher compared to control mice as it already reached 247± 15.5 μm when pressurized at 10 mmHg and increased up to approximately 290 μm at the maximal intraluminal pressure (Fig. 1a). Consequently, the compliance of TDAs isolated from high fat-fed mice was significantly lower compared to control TDAs for intraluminal pressure ranging from 10 to 70 mmHg (Fig. 1b).

When pressurized at 10 mmHg, the passive diameter measured in carotids isolated from control mice was slightly lower compared to carotids isolated from high fat-fed mice (236± 8.31 μm for control carotids and 287±7.18 μm for carotids isolated from high fat-fed mice, Fig. 1c). However, the increase in intraluminal pressure induced a similar increase of the passive diameter between control carotids and carotids isolated from high fat-fed mice (Fig. 1c). The compliance of control carotids was not different compared to compliance of carotids isolated from high fat-fed mice (Fig. 1d).

Histological Alterations of Carotid and TDA in High Fat-Fed Mice

Cross sections of TDAs and carotids isolated from control and high fat-fed mice were subjected to several histological stains. The H&E stain demonstrates that the TDAs are composed of three different layers: the intima on the luminal side, separated from a thin tunica media by an internal elastic lamina and a thick and fibrous adventitia on the outer side of the artery (Fig. 2a–d). The PAS staining of TDAs isolated form high fat-fed mice revealed an abundant magenta stain, which corresponds to the presence of glycoproteins in the arterial wall (Fig. 2e–h). Staining of TDA cross sections with Gömöri and Masson's trichromes allowed us to visualize connective tissues. Both stains showed the presence of a thicker adventitia with a denser blue stain as well as the presence of blue stain in the media tunica layer of TDAs isolated from high fat-fed

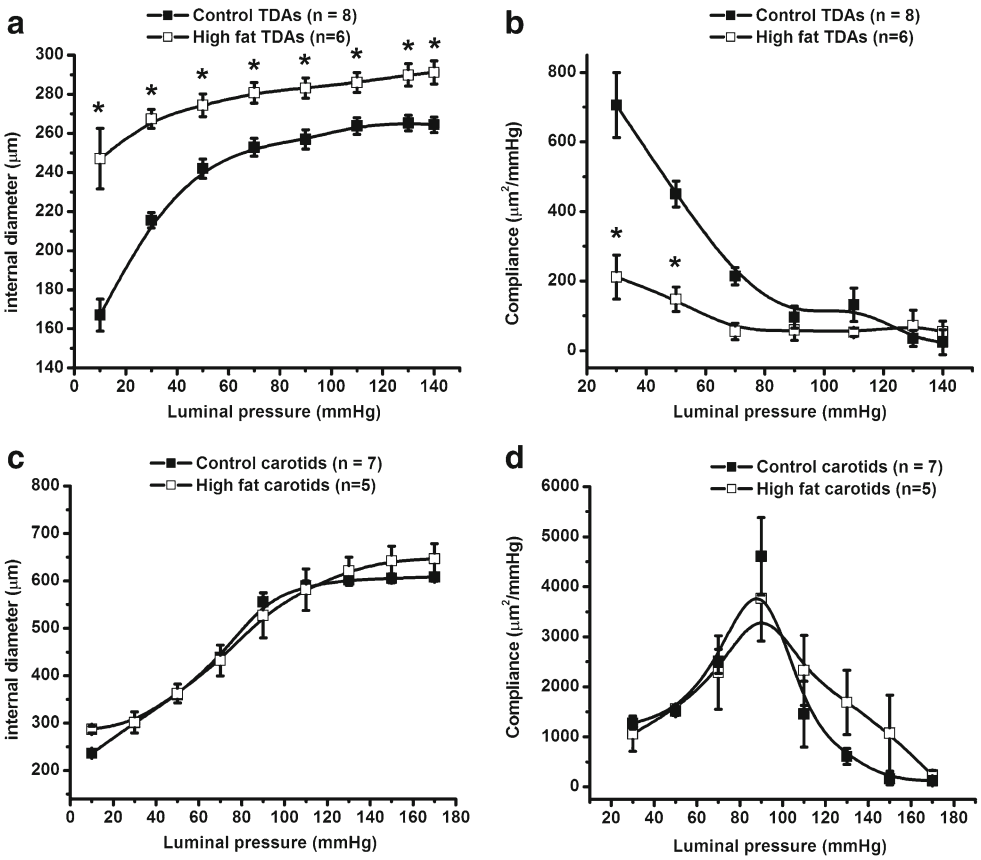

Fig. 1 Compliance of carotid and TDA in control and high fat-fed mice. The passive diameters developed by TDAs (**a**) and carotids (**c**) at increased intraluminal pressures were compared between control and high fat-fed mice. The data in **a** and **c** were used to calculate the compliance of TDAs (**b**) and carotids (**d**) isolated from controls and high fat-fed mice

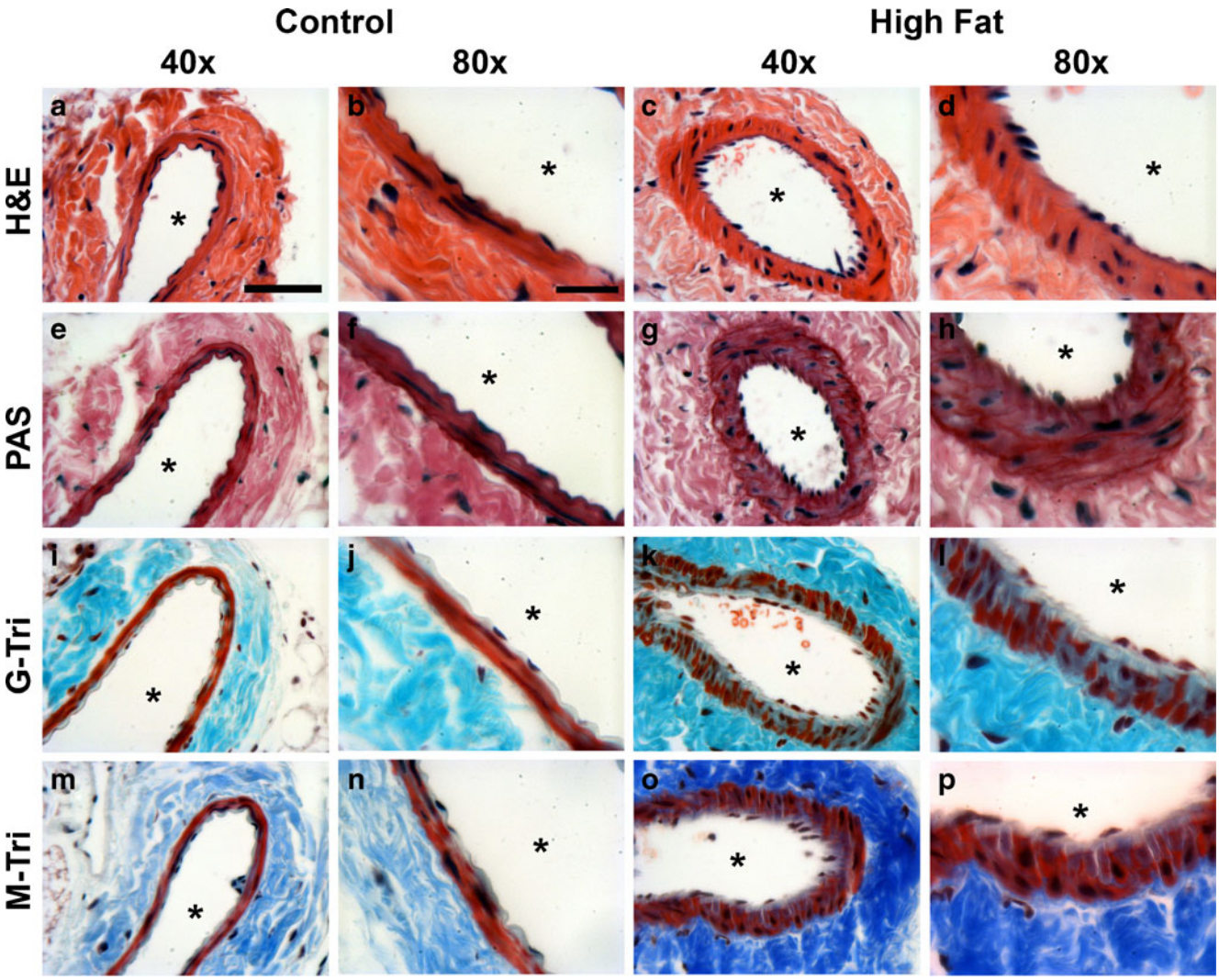

Fig. 2 Histological study of TDA in control and high fat-fed mice. Cross sections of TDAs isolated from control (*left panel*) and high fat-fed mice (*right panel*) were subjected to different stains: hematoxylin and eosin (H&E, **a**–**d**), periodic acid-Schiff (PAS, **e**–**h**), Gömöri trichrome (G-Tri, **i**–**l**), and Masson's trichrome (M-Tri, **m**–**p**) and observed with ×40 and ×80 magnifications. *Scale bar* is 50 μm and 20 μm in 40× and 80× magnifications respectively. *Asterisk* indicates the lumen

mice compared to control TDAs (Fig. 2i–p). This denser blue stain is a marker of collagen fibers demonstrating an abundant collagen deposition in TDAs isolated from high fat-fed mice.

The H&E stain of carotid transverse sections also revealed the presence of three different layers in the arterial wall (Fig. 3a–d). As observed for the TDAs, the PAS stain performed on carotids exhibited a denser magenta stain of the tunica media, revealing a prominent presence of glycoproteins in the carotid smooth muscle of high fat-fed mice (Fig. 3e–h). Similarly to TDAs, Gömöri and Masson's trichromes revealed the abundance of collagen fibers in the adventitia as well as between the smooth muscle cells in carotids isolated from high fat-fed mice compared to control carotids (Fig. 3i–p).

Subcellular Alterations of Carotids and TDAs in High Fat-Fed Mice

The transmission electron microscopy (TEM) technique allowed us to study the ultrastructure of the TDA and carotid wall with more precision and revealed structural alterations in arteries isolated from high fat-fed mice compared to control arteries. Indeed, TEM on TDAs isolated from high fat-fed mice revealed the presence of collagen deposition between smooth muscle cells, which is consistent with Gömöri and Masson's trichromes stains (Fig. 4a–d). The TEM performed on carotids isolated from high fat-fed mice showed that the morphology of the smooth muscle cells was dramatically altered (Fig. 4g, h). Similarly to observations made on TDAs, the carotids isolated from high fat-fed mice

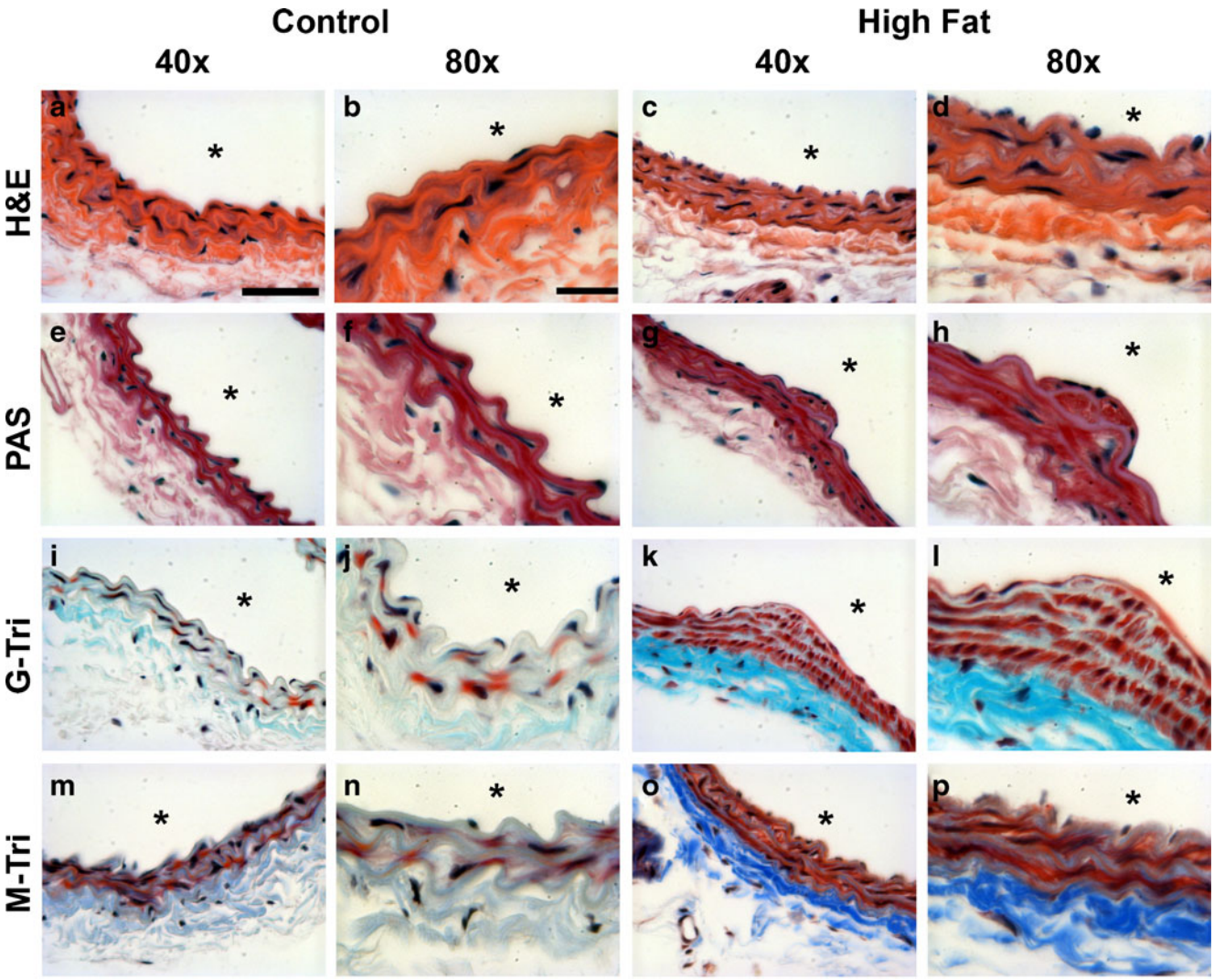

Fig. 3 Histological study of carotid in control and high fat-fed mice. Cross sections of carotids isolated from control (*left panel*) and high fat-fed mice (*right panel*) were subjected to different stains: hematoxylin and eosin (H&E, **a**–**d**), periodic acid-Schiff (PAS, **e**–**h**), Gömöri trichrome (G-Tri, **i**–**l**), and Masson's trichrome (M-Tri, **m**–**p**) and observed with ×40 and ×80 magnifications. *Scale bar* is 50 μm and 20 μm in 40× and 80× magnifications respectively. *Asterisk* indicates the lumen

had an abundant collagen deposition between the smooth muscle cells (Fig. 4e–h).

Discussion

Numerous studies have shown that obesity and more generally metabolic diseases are accompanied by profound alterations in large artery structure and function. These alterations include modifications of arterial wall components resulting in changes in arterial compliance and thus in stiffer carotids or aorta. Although there is an abundant literature regarding the alteration of carotid and aorta properties, there are very few studies on the characteristics of smaller arteries in metabolic diseases. Furthermore, because there is an abundant literature on severely obese animal model, this study has been conducted on a model of early stage obesity, induced by a short-term high fat diet (6 weeks) so as to determine the mechanisms involved in the development of cardiovascular alterations associated with obesity.

With increasing age or during metabolic diseases lipids accumulate and the intima of large arteries progressively thickens, which can lead to the development of atherosclerotic plaques [8, 12]. Thus, the arterial wall becomes less elastic and can lead to increased peripheral resistance and blood pressure in severe cases [4]. Also, it has been reported that the aortic elastic content of the tunica media decreases with age, leading to a decrease in vessel compliance; however, the contribution of aging to aortic stiffness is relatively minor compared with the influence of atherosclerosis [8]. The increase in vascular

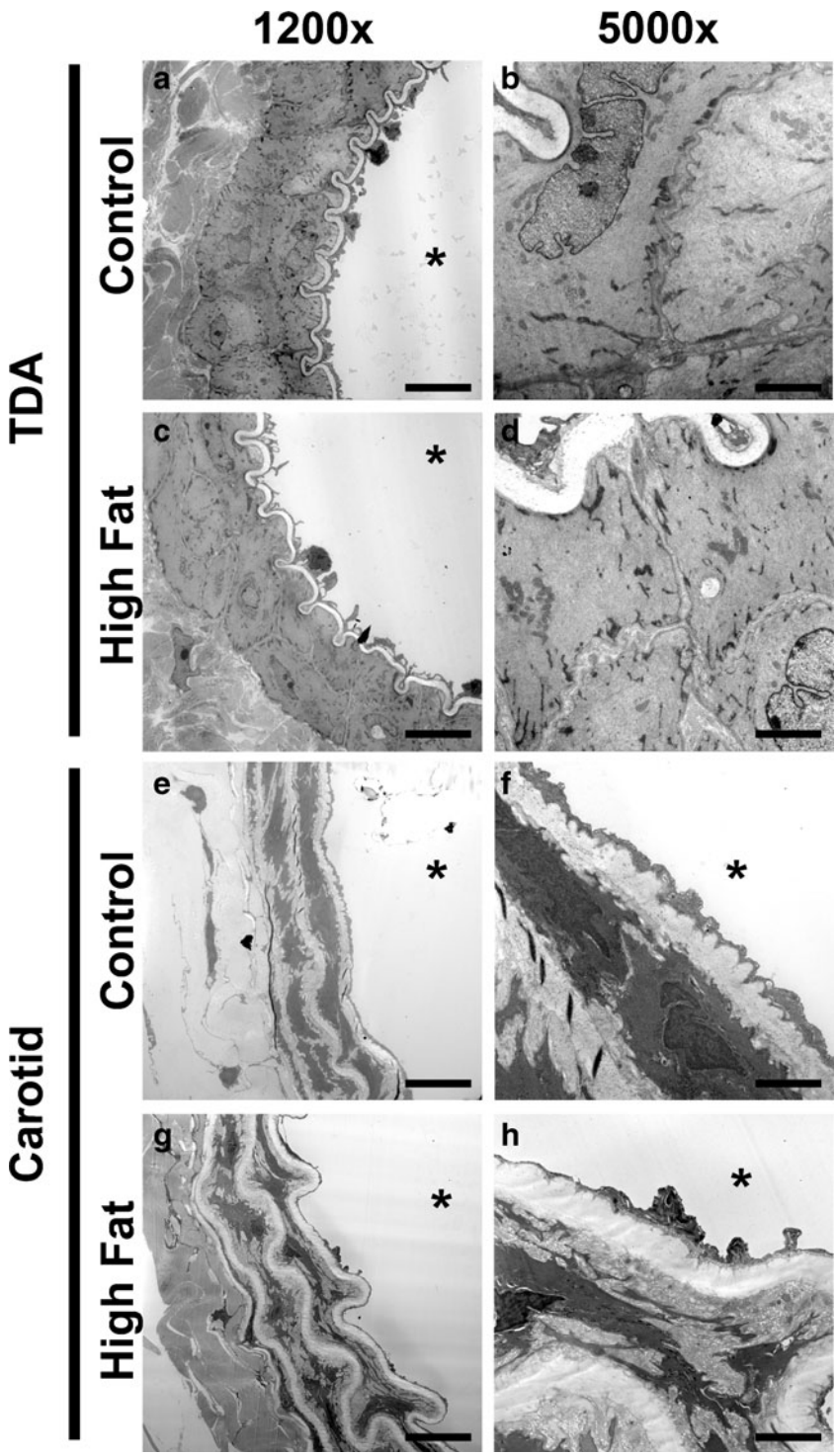

Fig. 4 Subcellular study of the structure of carotid and TDA arterial wall in control and high fat-fed mice. Representative transmission electron microscopy images of TDAs (**a**–**d**) and carotids (**e**–**h**) isolated from control (**a**, **b**, **e**, **f**) and high fat-fed mice (**c**, **d**, **g**, **h**) and observed at ×1,200 and ×5,000 magnifications. *Asterisk* indicates the lumen. *Scale bar* in **a**, **c**, **e**, and **g** is 10 and 2.5 μm in **b**, **d**, **f**, and **h**

stiffness observed with aging in large arteries has also been reported in smaller arteries [9, 13]. For all these reasons, animals used in our study were the same age in the control and high fat-fed groups.

In this study, we show that the TDAs from high fat-fed mice have a larger internal lumen diameter at each intraluminal pressure as compared to TDAs from control mice. These results are consistent with a previous report showing an increase in lumen diameter (approximately 75%) of the 4th branch of the superior mesenteric artery in a mouse model of type 2 diabetes compared to control [7]. Furthermore, mechanical study of TDA from high fat-fed mice showed almost a complete loss in arterial compliance demonstrating a stiffening of the TDA in pathological condition. This stiffening

was accompanied by several histological modifications of the TDA arterial wall such as an increase in collagen deposition as demonstrated by Gömöri and Masson's trichrome as well as by TEM. Although the same histological modifications could be observed in the carotid isolated from high fat-fed mice, the carotid lumen diameter and the carotid compliance remained unchanged.

The components of the ECM are an important determinant of the arterial wall integrity and arterial compliance. In smaller arteries such as the TDA, the internal elastic lamina and the adventitia are enriched in fibrous components such as collagen and elastin, which respectively provide tensile strength and elasticity. In larger arteries such as the carotid, there are additional layers of fibrous tissue within the ECM between the different smooth muscle cell layers providing a higher elasticity compared to smaller arteries [14]. The elasticity of the arterial wall is essential for expansion and recoil during the systolic/diastolic cycles of the heart, especially in the large arteries close to the heart. Thus, alterations of the mechanical properties of arteries can lead to severe cardiovascular dysfunctions.

Although the mechanisms initiating arterial remodeling in metabolic diseases are poorly understood, one study conducted on a mouse model of type 2 diabetes demonstrated that there is an imbalance between the enzymes that degrade the matrix (the matrix metalloproteinases, MMPs) and their inhibitors (the tissue inhibitors of matrix metalloproteinase, TIMPs), which could lead to excess ECM accumulation [7]. Similarly, another regulator of ECM dynamics, the plasminogen activator inhibitor-1 is increased in metabolic diseases and is also considered a marker of several metabolic diseases such as obesity and type 2 diabetes [7]. These observations have also been reported in diabetic patients who exhibit marked increases in MMP and TIMP plasma and urine concentrations [15, 16]. In addition to changes in the fibrous components of the ECM, the PAS stains performed on TDAs and carotids isolated from high fat-fed mice revealed an increased presence of glycoproteins in the ECM. These results are consistent with multiple reports showing an accumulation of advanced glycation end products in metabolic diseases [17]. In our model, the arterial remodeling observed (e.g., increase in collagen) is most probably due to an alteration in the dynamic regulation of the ECM; however, the molecular mechanisms underlying these alterations in high fat-fed mice requires further investigation.

Arterial remodeling is commonly observed in small arteries in several pathologies, including metabolic diseases, and this remodeling appears to be the fundamental hemodynamic abnormality contributing to increased peripheral resistance and, in the long term, to increased blood pressure [5]. These changes include a thickening of the arterial wall inducing a reduction of the luminal diameter, which directly results in an increase in peripheral resistance [5]. Despite the lack of study on the relation between arterial stiffening and remodeling of small arteries in obesity, there are several reports on other pathological models, which can relate to some extent to metabolic diseases. For example, the mouse model of angiotensin II-induced hypertension could relate to metabolic diseases as there are several lines of evidence suggesting an activation of the renin–angiotensin system resulting in increased circulating angiotensin II in metabolic diseases [18]. In these mice, an increase in collagen was observed along with a stiffening of the 2nd order of the mesenteric artery and an overall increase of blood pressure [19, 20]. Altogether, this suggests that the remodeling observed in our model may be a first step which could lead to a more severe vascular remodeling with a thickening of the media, reducing the lumen diameter and resulting in increased peripheral resistance and blood pressure.

Although the presence of collagen deposits could be observed in carotids from high fat-fed mice, we did not measure any decrease of carotid compliance. This is consistent with observation on a mouse model of atherosclerosis (ApoE KO mice fed with an atherogenic diet) where the presence of histological alterations in the aorta (presence of plaque, media thickening, and disruption of elastin fibers) is observed earlier than the stiffening of the aorta, which only appears at 34 weeks of age [8]. This suggests that our high fat-fed mouse model corresponds to an early stage of obesity with a low degree of structural remodeling.

In summary, our study shows a significant increase of the lumen diameter accompanied by a significant decrease of arterial wall compliance in TDAs from high fat-fed mice whereas no changes in lumen diameter and arterial compliance could be measured in carotids from high fad-fed mice. These results correlated with alterations in the TDA arterial wall structure as the TDAs isolated from high fat-fed mice exhibited an increase in collagen staining which was confirmed by the presence of collagen deposition observed using TEM. These results suggest that, at early stage of obesity, the structural properties of small and large arteries are altered whereas arterial stiffness is only observed in small vessels implying that small vessels are targeted earlier compared to large arteries. The early targeting of small vessels could therefore play a role in the development of hypertension associated to a long-term caloric-enriched diet.

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