Regional variation in arterial stiffening and dysfunction in Western diet-induced obesity

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1Research Service, Harry S. Truman Memorial Veterans’ Hospital, Columbia, Missouri; 2Department of Biomedical Sciences, University of Missouri School of Medicine, Columbia, Missouri; 3Dalton Cardiovascular Research Center, University of Missouri School of Medicine, Columbia, Missouri; 4Department of Biological Engineering, University of Missouri, Columbia, Missouri; 5Department of Medicine-Endocrinology, Diabetes and Metabolism University of Missouri School of Medicine, Columbia, Missouri; 6Department of Medical Pharmacology and Physiology, University of Missouri School of Medicine, Columbia, Missouri; and 7School of Mathematics, Computer Science and Engineering, City University London, London, United Kingdom

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Bender SB, Castorena-Gonzalez JA, Garro M, Reyes-Aldasoro CC, Sowers JR, DeMarco VG, Martinez-Lemus LA. Regional variation in arterial stiffening and dysfunction in Western diet-induced obesity. Am J Physiol Heart Circ Physiol 309: H574–H582, 2015. First published June 23, 2015; doi:10.1152/ajpheart.00155.2015.—Increased central vascular stiffening, assessed in vivo by determination of pulse wave velocity (PWV), is an independent predictor of cardiovascular event risk. Recent evidence demonstrates that accelerated aortic stiffening occurs in obesity; however, little is known regarding stiffening of other disease-relevant arteries or whether regional variation in arterial stiffening occurs in this setting. We addressed this gap in knowledge by assessing femoral PWV in vivo with ex vivo analyses of femoral and coronary structure and function in a mouse model of Western diet (WD; high-fat/high-sugar)-induced obesity and insulin resistance. WD feeding resulted in increased femoral PWV in vivo. Ex vivo analysis of femoral arteries revealed a leftward shift in the strain-stress relationship, increased modulus of elasticity, and decreased compliance indicative of increased stiffness following WD feeding. Confocal and multiphoton fluorescence microscopy revealed increased femoral stiffness involving decreased elastin/collagen ratio in conjunction with increased femoral transforming growth factor-β (TGF-β) content in WD-fed mice. Further analysis of the femoral internal elastic lamina (IEL) revealed a significant reduction in the number and size of fenestrae with WD feeding. Coronary artery stiffness and structure was unchanged by WD feeding. Functionally, femoral, but not coronary, arteries exhibited endothelial dysfunction, whereas coronary arteries exhibited increased vasoconstrictor responsiveness not present in femoral arteries. Taken together, our data highlight important regional variations in the development of arterial stiffness and dysfunction associated with WD feeding. Furthermore, our results suggest TGF-β signaling and IEL fenestrae remodeling as potential contributors to femoral artery stiffening in obesity.

pulse wave velocity; femoral; coronary; confocal microscopy

NEW & NOTEWORTHY

Our data reveal differential development of conduit arterial stiffening and vasomotor dysfunction between femoral and coronary arteries in Western diet (WD)-induced obesity. Increased femoral stiffness in vivo following WD feeding is associated with endothelial dysfunction, aberrant extracellular matrix accumulation, increased transforming growth factor-β content, and internal elastic lamina fenestrae remodeling.

CARDIOVASCULAR DISEASE (CVD) is a leading cause of death in patients with obesity, insulin resistance, and diabetes. Recent analysis of the Framingham Offspring Study demonstrated that stiffening of large conduit blood vessels (i.e., arterial stiffening) is an early vascular defect that is associated with and precedes the development of hypertension (22). Importantly, increased central vascular pulse wave velocity (PWV), a measure of arterial stiffening, is independently associated with CVD event risk (31). Accelerated arterial stiffening occurs in obesity whereby increased body mass index is associated with reduced arterial distensibility as early as adolescence (39, 49, 51) and is associated with angiographic evidence of coronary artery disease later in life (3). Thus, arterial stiffening is a significant contributor to and predictor of CVD development and progression.

While central measures of vascular stiffness (i.e., carotid-femoral/aortic PWV) predict CVD risk and mortality, it has recently been suggested that delineation of local vascular stiffness may provide more clinical value with regard to CVD outcomes (34). This assertion is based, in part, on structural differences between conduit arteries (elastic versus muscular arteries) and the inherent differences in pathophysiology of vascular stiffness. The importance of regional stiffness measures was recently validated in a post hoc analysis of the population-based Hoorn study (47). This landmark analysis demonstrated that local measures of carotid and femoral artery stiffness were independently associated with CVD events and all-cause mortality (47). Importantly, these measures were predictive independently of each other and of central PWV. Whether local stiffening of conduit arteries can ultimately predict vascular bed-specific outcomes remains to be determined; however, these data have led to speculation as to whether femoral artery stiffening in particular may serve as a surrogate for conduit coronary artery stiffening due to their similar makeup (47). Accumulating evidence demonstrates that the accelerated stiffening of large conduit arteries (primarily the aorta) in obesity involves vascular remodeling processes including vascular cell proliferation, migration, hypertrophy, and changes in the composition and makeup of the extracellular matrix. Re-
cent studies have described a number of specific processes underlying obesity-associated aortic stiffening and increased PWV, including intima-medial thickening with fibrosis (13), medial (i.e., smooth muscle) hypertrophy with increased extracellular matrix cross-linking (48), and increased aortic collagen content and disorganization (37). Consistent with other reports, these changes often occur in concert with obesity-associated aortic endothelial dysfunction and inflammation (38, 48). Thus, aortic stiffening in obesity has become well established and appears to involve various mechanisms throughout the vessel wall. Whether similar changes are associated with stiffening of other regional conduit or muscular arteries in obesity remains undetermined as does the relationship of local stiffening with vascular cell and vasomotor dysfunction, an additional early marker of CVD.

We directly address this gap in knowledge in the present study by examining the hypothesis that feeding of a high-fat, high-sugar Western diet (WD) would induce femoral and coronary artery stiffening and dysfunction in conjunction with increased femoral PWV. We examined this hypothesis via in vivo determination of PWV coupled with ex vivo arterial function measurements and structural assessment utilizing confocal and multiphoton imaging of the vascular wall.

**METHODS**

**Animals.** All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Missouri and Harry S. Truman Veterans’ Memorial Hospital. Male C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) were given ad libitum access to water and either a WD (TestDiet 5APC modified, St. Louis, MO) consisting of 46% fat and 36% carbohydrate (17.5% each from sucrose and high-fructose corn syrup) or a normal control diet (Con; TestDiet 5APD) for 16 wk beginning at 4 wk of age. Animals were housed in pairs in a temperature-controlled facility on a 12:12-h light-dark cycle. On the day of euthanasia, mice were fasted for 5 h, anesthetized with isoflurane (2–4% in 100% O2), and euthanized by exsanguination following blood/tissue collection. Plasma glucose was determined by glucometer, and other plasma parameters were determined by an outside laboratory (Comparative Clinical Pathology Service, Columbia, MO).

In vivo femoral pulse wave velocity. Doppler ultrasound (Indus Mouse Doppler System, Webster, TX) was performed on male mice the week prior to their being euthanized according to a previously established protocol (19) to evaluate PWV, the gold standard technique for in vivo determination of arterial stiffness. Calculation of PWV is based on the transit time method utilized to determine the difference in arrival times of a Doppler pulse wave at two locations along an artery a known distance apart. Arrival time of each pulse wave is measured as the time from the peak of the ECG R-wave to the foot of the pulse wave at which time velocity begins to rise at the start of systole. The distance between the two locations along the blood vessel is divided by the difference in arrival times and is expressed in millimeters per second. An increase in PWV corresponds to stiffening of the vessel. All Doppler procedures were performed on isoflurane-anesthetized mice (1.75% in 100% O2). Doppler pulse waves were measured rapidly in series at the iliac bifurcation and at the femoral artery (1 cm downstream). Femoral artery PWV was calculated for assessment of arterial stiffness.

Vascular function and structure. Vascular endpoints were assessed in femoral and coronary arteries collected from the same animal.

**Femoral artery.** Proximal femoral arteries were collected from both left and right lower limbs and immediately placed in cold (~4°C) physiological saline solution (PSS) containing (in mM): 145.0 NaCl, 4.7 KCl, 2.0 CaCl2, 1.0 MgSO4, 1.2 NaH2PO4, 0.02 EDTA, 2.0 pyruvic acid, 5.0 glucose, and 3.0 MOPS (pH 7.4). A section of each femoral was isolated from surrounding tissue, cannulated, and pressurized for experimentation as previously described (29). Briefly, artery segments were cannulated in observation chambers (Living Systems Instrumentation, Burlington, VT) and pressurized without flow to 70 mmHg with PSS containing 0.15 mM bovine serum albumin using a Pressure Servo System (Living Systems Instrumentation, Burlington, VT), with each vessel visualized on an inverted microscope. Artery wall thickness and diameter were determined using video calipers (Living Systems Instrumentation). After warming to 37°C and equilibration, vessel viability was confirmed by vasoconstriction to PSS containing 80 mM KCl (equimolarly substituted for NaCl). Following washout, adrenergic vasoconstrictor responses to phenylephrine (PE; 10−6–10−3 M) were determined by cumulative addition of agonist to the bath. Subsequently, vasodilator responses to acetylcholine (ACh; 10−8–10−4 M) or insulin (10−9–10−5 M), and sodium nitroprusside (SNP; 10−5–10−4 M) were determined after vessels were preconstricted with 10−4 M PE. Maximal passive diameter was obtained at the end of each experiment by replacing the PSS with calcium-free PSS containing 2 mM EGTA and 10−4 M adenosine. Vasoconstrictor responses are reported as percent maximal diameter, and vasodilator responses are reported as percent dilation from PE preconstriction. Elastic characteristics of the vessel wall were determined under passive (i.e., calcium-free) conditions in buffer containing 2 mM EGTA and 10−4 M adenosine. Step changes in intraluminal pressure (5–120 mmHg) were performed, and internal diameter and wall (left and right) thicknesses were recorded at each pressure. These data were subsequently used to calculate circumferential stress, strain, cross-sectional compliance, and modulus of elasticity curves for each group of vessels, as previously described (11, 12, 42).

**Coronary artery.** The heart was collected and immediately placed in cold Krebs PSS containing (in mM): 119.0 NaCl, 4.7 KCl, 2.5 CaCl2, 1.17 MgSO4·7H2O, 25 NaHCO3, 1.18 KH2PO4, 0.027 EDTA, and 5.5 Glucose (pH 7.4). Segments of the left coronary artery (1 mm long) were carefully dissected and mounted on a 17-μm stainless steel wires in oxygenated Krebs PSS (95% O2-5% CO2) in a small vessel myograph for isometric tension recording (Danish Myo Technology, Aarhus, Denmark). Wire myography was utilized for the coronary artery due to difficulties in obtaining adequately long artery segments without branches, as required for pressure myography. Vessel length was measured after mounting with a calibrated lens in the dissection scope. After warming to 37°C and equilibration, normalization was performed as previously described (35), and vessels were stretched to achieve an internal circumference corresponding to a transmural pressure of 90 mmHg. Vessel viability was subsequently assessed by exposure to 80 mM KCl Krebs PSS. Following washing, vasodilator responses to ACh (10−9–10−4 M), insulin (0.1–300 ng/ml), and SNP (10−9–10−4 M) were assessed by cumulative addition of agonist to the bath following preconstriction with the thromboxane A2 analog U46619 (0.1–300 μM). U46619 was used as a preconstrictor, since preliminary experiments revealed that isolated mouse coronary arteries do not respond to PE (data not shown). Similarly, in a separate vessel segment, vasoconstrictor responses were assessed to U46619 (10−8–10−6 M). Minimum tension was determined at the end of the experiment by replacing the Krebs PSS with calcium-free Krebs PSS. Vasoconstractor responses are reported as a percent maximal dilation from U46619 preconstriction. Vasoconstrictor responses are reported as developed tension normalized to vessel length (mN/mm). Since these isolated vessels do not develop spontaneous myogenic tone, unlike smaller mouse coronary arterioles (2, 32), and no difference was present between minimum tension (in calcium-free Krebs PSS) and the initial tension at mounting, elastic properties were assessed by the circumference-tension curve generated during the normalization procedure.

**Confocal/multiphoton fluorescence microscopy imaging.** At the end of each experimental protocol, femoral and coronary artery
segments were fixed in 4% paraformaldehyde. Femoral arteries were fixed while pressurized at 70 mmHg. Vessels were then rinsed twice in phosphate-buffered saline (PBS) and once in 0.1 M glycine (5 min each). Cannulated femoral arteries were flushed with 1 ml of PBS and then permeabilized with 0.5% Triton X-100 for 20 min followed by washing with PBS and incubation with 0.5 μg/ml 4',6-diamidino-2-phenylindole (DAPI), 0.2 μM Alexa Fluor 633 hydrazide (Molecular Probes), and 0.02 μM Alexa Fluor 546 phallidin (Molecular Probes) in PBS for 1 h. Following incubation, vessels were washed in PBS three times and imaged using a Leica SP5 confocal/multiphoton microscope with a 63x/1.2 numerical aperture water objective. Alexa Fluor 633 was used to stain and image elastin and was excited with a HeNe laser at 633 nm (14). Alexa Fluor 546 phallidin was used to stain and image actin fibers and was excited with a HeNe laser at 543 nm (43). DAPI was used to stain and image nuclei and was excited with a multi-photon laser at 720 nm (43). Collagen was imaged using a multi-photon laser at 850 nM via second-harmonic image, a scattering nonlinear process that requires no staining (30). Images were processed, and all channels were quantified to determine the total volume (number of expressed voxels) occupied by vascular smooth muscle cell (VSMC) nuclei, elastin, actin, (within the media), and collagen with an in-house MATLAB algorithm available upon request. Further analysis of the elastin channel with ImageJ was performed to quantify and characterize the fenestration of the IEL.

Immunohistochemistry. The expression of total TGF-β was quantified by immunohistochrometry on paraffin-embedded sections of femoral arteries fixed in 4% paraformaldehyde. Briefly, paraffin-embedded arterial sections (5 μm) were deparaffinized and rehydrated via immersion in xylene and in a series of ethanol solutions (100%, 95%, and 70%). A 10 mM sodium citrate buffer was used for antigen retrieval, and sections were blocked with an SSC (saline sodium citrate) antibody-buffer containing 1% BSA and 0.05% Triton for 30 min and then incubated overnight with rabbit polyclonal anti-TGF-β (Abcam AB66043; 1:500 dilution in SSC antibody-buffer) at 4°C. Sections were thoroughly washed (4 × 10 min each) with a SSC antibody-wash-buffer containing 0.5% Triton and incubated with secondary antibody goat anti-rabbit IgG(H+L) Alexa 488 (Invitrogen A11034, 1:1,000 dilution) for 1 h at room temperature. After a washing, sections were mounted with Prolong Diamond Antifade Mountant with DAPI (Molecular Probes P36962) for further examination under a confocal microscope (Leica SPE) using 405- and 488-nm laser lines. All images were obtained using identical excitation laser power and acquisition gain. Signal intensities were analyzed with ImageJ. Results are expressed as total expression of TGF-β normalized to the total area expressing the protein (in pixels).

Plasma TGF-β determination. Plasma levels of TGF-β1, -β2, and -β3 were determined by multiplex assay (Bio-Plex Pro, Bio-Rad), according to the manufacturer instructions.

Statistics. Data are presented as means ± SE. Statistical analysis was performed by Student’s t-test, two-way ANOVA followed by Bonferroni post hoc test, or Pearson correlation, as appropriate. A P value of <0.05 was considered significant.

RESULTS

WD feeding induced obesity and insulin resistance in C57BL6/J mice. Similar to our previous reports (8, 33), male mice fed a WD for 16 wk were obese with epididymal fat pad expansion and had elevated plasma glucose, insulin, and cholesterol levels and insulin resistance, assessed by HOMA-IR, compared with those fed a control diet (Table 1).

Femoral PWVs were increased by WD feeding. Femoral PWV was assessed by Doppler ultrasound. Compared with control mice, mice fed a WD for 16 wk exhibited a 63% increase in femoral PWV (Fig. 1). Heart rate was not different between control (458 ± 9 beats/min) and WD fed (452 ± 9 beats/min) mice. We had previously published no change in mean or diastolic blood pressure in WD-fed male mice, the latter being a primary determinant of PWV (21).

WD feeding induced femoral, but not coronary, artery endothelial dysfunction. Endothelial function of femoral and coronary arteries was assessed using pressure and wire myography, respectively. There were no differences in passive diameters of femoral (282 ± 6 vs. 270 ± 6 μm) or coronary (259 ± 5 vs. 263 ± 6 μm) arteries from control and WD-fed mice, respectively. Vasodilation to the endothelium-dependent vasodilator ACh was reduced in femoral, but not coronary, arteries from WD-fed mice compared with control mice (Fig. 2A), whereas WD feeding had no effect on insulin-induced vasodilation in either treatment group (Fig. 2B). Dilation to the NO donor SNP was unchanged in either group, demonstrating normal smooth muscle NO sensitivity (Fig. 2C).

WD feeding increased coronary, but not femoral, artery vasomotor responsiveness. Vasoconstrictor responses of pressurized femoral arteries to the α1-adrenergic agonist PE (Fig. 3A) and 80 mM KCl were unchanged in WD-fed mice compared with control mice. Percent constriction to 80 mM KCl in femoral arteries was 35 ± 6% in control and 33 ± 4% in WD mice. Conversely, WD feeding increased coronary artery constriction to the thromboxane A2 analog U46619 (Fig. 3B) but not to 80 mM KCl compared with control. Coronary artery developed tension to 80 mM KCl was 2.6 ± 0.2 mN/mm in control and 2.9 ± 0.1 mN/mm in WD mice.

WD feeding induced femoral, but not coronary, artery stiffening and IEL remodeling. In WD mice, the femoral artery strain-stress relationship was significantly left-shifted (Fig. 4A), whereas the coronary artery circumference-tension (equivalent to passive length-tension) relationship was not changed (Fig.

Table 1. Phenotypic characteristics of control and Western diet-fed mice

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Western Diet</th>
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<tr>
<td>Body weight, g</td>
<td>27.8 ± 0.3</td>
<td>38.8 ± 1.3*</td>
</tr>
<tr>
<td>Heart weight/tibia length, mg/cm</td>
<td>0.35 ± 0.02</td>
<td>0.30 ± 0.02†</td>
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<tr>
<td>Epididymal fat pad weight, g</td>
<td>0.68 ± 0.03</td>
<td>2.25 ± 0.11*</td>
</tr>
<tr>
<td>Plasma glucose, mg/dl</td>
<td>139 ± 4</td>
<td>182 ± 3*</td>
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<tr>
<td>Plasma insulin, ng/ml</td>
<td>0.22 ± 0.002</td>
<td>0.28 ± 0.014*</td>
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<tr>
<td>HOMA-IR</td>
<td>1.38 ± 0.06</td>
<td>2.22 ± 0.13*</td>
</tr>
<tr>
<td>Plasma cholesterol, mg/dl</td>
<td>127 ± 4</td>
<td>151 ± 6*</td>
</tr>
<tr>
<td>Plasma triglycerides, mg/dl</td>
<td>39 ± 5</td>
<td>51 ± 6</td>
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Values are means ± SE; n = 8–10. *P < 0.05 vs. control; †P = 0.09 vs. control.

Fig. 1. Western diet feeding increases femoral pulse wave velocity in vivo. Mice were fed control (Con) or Western diet (WD) for 16 wk prior to pulse wave velocity (PWV) determination at the approximate sites shown in schema on right. Fem, femoral. Values are means ± SE; n = 6–7. *P < 0.05 vs. Con.
compared with control mice. Additional analysis revealed that the femoral artery pressure-diameter relationship, an index of remodeling, was unchanged in WD mice (Fig. 5A), whereas the femoral modulus of elasticity, an index of stiffness, was increased (Fig. 5B), indicating WD-associated femoral artery stiffening. Cross-sectional compliance, an additional measure of stiffness that is often independent of distensibility measures, was also determined. Femoral arteries from WD-fed mice were stiffer, i.e., had lower cross-sectional compliance, than control mice at an intravascular pressure of 40 mmHg (Fig. 5C). Confocal and multi-photon fluorescence microscopy was used to further assess the composition of the vascular wall in femoral and coronary arteries. These experiments revealed no significant changes in the number of voxels expressing actin and nuclei in the media, or elastin and collagen in the femoral artery wall (Fig. 6A). This was unchanged when normalized to volume (μm³, actin and nuclei) or area (μm², elastin and collagen; data not shown). The ratios of normalized actin/nuclei and elastin/collagen were significantly reduced in femoral arteries from WD-fed mice (Fig. 6A). Media thickness was similar between femoral arteries from control (13 ± 0.5 μm) and WD (13 ± 0.8 μm) mice. Imaging of the coronary artery wall revealed no changes in the number of voxels expressing actin, nuclei, elastin, or collagen (also similar when normalized as reported for the femoral artery), and unchanged actin/nuclei and elastin/collagen ratios (Fig. 6B). Coronary media thickness was also similar between control (10 ± 0.8 μm) and WD (12 ± 2.2 μm) mice. Additional analysis of the IEL revealed that WD-fed animals exhibited a reduction in the number of femoral artery fenestrae, the mean fenestra size, and subsequently the total area occupied by fenestrae in the IEL compared with control mice (Fig. 7). Coronary arteries from control and WD animals exhibited similar number and size of fenestrae in the IEL between control and WD-fed mice (data not shown).

WD feeding increased femoral, but not circulating, TGF-β levels. Immunohistochemistry revealed a more than twofold increase in total TGF-β in femoral arteries from WD-fed mice compared with control (Fig. 8). Pearson correlation analysis revealed significant positive correlations between femoral total TGF-β content and modulus of elasticity at 120 mmHg (Pearson coefficient 0.66, $P < 0.05$) and femoral collagen (Pearson coefficient 0.64, $P < 0.05$). Conversely, plasma levels of TGF-β1–3 assessed by multiplex assay were unchanged. Plasma levels of TGF-β1 were 12.9 ± 4.9 and 7.9 ± 3.0 μg/ml in control and WD mice, respectively ($P > 0.05$). Similarly, plasma levels of TGF-β2 were not different between control (247 ± 31 pg/ml) and WD (224 ± 36 pg/ml). Plasma levels of
TGF-β3 were unchanged in WD (23 ± 11 pg/ml) compared with control (58 ± 19 pg/ml) mice (P = 0.07).

**DISCUSSION**

Accelerated central vascular stiffening has become an established component of obesity-related cardiovascular disease. A primary finding of the present study was that endothelial vasodilator dysfunction and stiffening induced by WD feeding occurred in the femoral, but not the coronary, artery. Specifically, WD feeding for 16 wk in male mice caused femoral endothelial vasodilator dysfunction and increased femoral PWV and ex vivo stiffness associated with IEL remodeling and increased TGF-β content. These data suggest that use of the femoral artery as a surrogate for the coronary artery in mice is inappropriate. WD feeding did, however, modestly increase coronary vasoconstrictor responsiveness. Taken together, our data reveal regional effects of WD feeding on vascular structure and function, and suggest increased TGF-β signaling and IEL remodeling as potential mechanisms associated with femoral stiffening in this model. To our knowledge, this is the first side-by-side comparison of femoral and coronary artery structure and function in a model of WD-induced obesity.

**Vascular bed-specific effects of WD on arterial stiffness.** Our data reveal a nonuniform distribution of increased stiffness and remodeling following WD feeding for 16 wk in that the femoral, but not coronary, artery becomes stiffer. Indeed, the WD-induced increase in femoral PWV in vivo in this study is similar in magnitude to that previously reported in a similar model in the aorta (48). Recent investigations of mechanisms underlying obesity-related increases in aortic stiffness have revealed a primary role for alterations in extracellular matrix composition and cross-linking. Specifically, increased aortic collagen content following high-fat diet feeding (37) and increased extracellular matrix cross-linking following high-fat/high-sucrose diet feeding (48) have been reported. Importantly, in the latter study, these changes occurred prior to the development of hypertension (48). In this study, confocal and multi-photon fluorescence imaging revealed no change in femoral media thickness and a decrease in the elastin-to-collagen ratio often characteristic of increased vascular stiffness. There-
fore, some characteristics of femoral stiffening (i.e., altered extracellular matrix composition) are consistent with mechanisms contributing to increased aortic stiffness in obesity.

Further analysis of the IEL, however, revealed the novel finding that WD feeding induced ultrastructural remodeling of fenestrae in the femoral IEL, resulting in fewer, smaller fenestrae. No matrix or IEL remodeling was found in the coronary artery following WD feeding. The IEL is a critical determinant of the mechanical properties of large arteries, and fenestrae, in addition to their role in cell-cell communication and permeability, play an important role in the ability of the IEL to bear circumferential wall stress. In particular, the stress-concentration phenomena dictate that stresses are elevated (concentrated) in the vicinity of holes in solid structures under tension and these stresses are concentrated less at smaller holes. Reduced carotid artery fenestra number and size have been reported in spontaneously hypertensive rats prior to the onset of hypertension (1, 9) and in rabbits after chronic flow reduction (50). Conversely, carotid fenestra enlargement has been reported following chronic reductions of longitudinal tension (20). These data suggest that hemodynamic and mechanical forces may be dynamic regulators of IEL ultrastructure and stability. Our observation that femoral cross-sectional compliance at 40 mmHg was decreased in mice fed a WD further suggests that arterial wall stiffness was increased even at lower intravascular pressures. Compliance is a more sensitive measure of stiffness at lower pressures and takes into account the tubular form of the vessel (26, 36, 42). In addition, vascular wall components such as elastin and cytoskeletal structures rather than collagen are more important contributors to the elastic properties of the vessel at lower intravascular pressures (42, 46). Whether the increased femoral stiffness associated with WD feeding involved the structural changes we observed in the IEL remains to be determined. Although we did not observe any changes in medial layer actin content, we cannot rule out the possibility that structural modification of the cytoskeleton contributed to the stiffening of femoral arteries in WD-fed mice, as other reports suggest that changes in the cytoskeleton of vascular smooth muscle contribute to vascular stiffening in aging and hypertension (40, 41).

Elastin and collagen production and stiffening of extracellular matrices have been associated with increased TGF-β signaling (15, 25, 27). Our data are consistent with this and demonstrate importantly that local femoral, not systemic circulating, TGF-β content is elevated by WD feeding and correlates with femoral collagen and stiffness, assessed by modulus of elasticity. Previous work has demonstrated that WD-associated insults, specifically high glucose and angiotensin II, stimulate vascular smooth muscle TGF-β1 signaling, collagen 1 release, and vascular stiffening in vivo (4). In addition, cross-talk from TGF-β1-treated smooth muscle cells to preferentially promote collagen 3 secretion from adventitial fibroblasts has recently been described (18). Thus, future studies are necessary to mechanistically delineate the specific TGF-β and collagen isoforms involved in WD-induced femoral stiffening. In addition, whether TGF-β signaling is involved in femoral IEL reorganization/remodeling warrants further examination, as TGF-β is a known stimulus for elastin production (27).

The lack of coronary structural remodeling in response to WD feeding in the present study was somewhat surprising based on evidence from other studies. Specifically, inward hypertrophic remodeling and reduced stiffness (due to elastin accumulation) of coronary arterioles has been described in db/db mice (23) and obese Ossabaw swine (44). In agreement with the present results, the former study demonstrated increased femoral PWV in db/db mice (23). Likewise, the latter study demonstrated increased stiffness of the left anterior descending coronary artery in obese swine (44). Thus, these studies highlight disparate effects of obesity on macrovascular and microvascular stiffness that may underlie the lack of coronary remodeling in the present study, as we examined the main coronary artery in the mouse rather than the coronary microcirculation. Furthermore, it should be noted that coronary structural remodeling occurs coincidentally with impaired cor-

Fig. 7. WD feeding induced femoral artery internal elastic lamina (IEL) remodeling. Confocal imaging was utilized to quantify IEL fenestra number, size, and area in femoral arteries following 16 wk of Con or WD feeding. Representative images on right were equally contrast enhanced for better print visualization of IEL fenestrae. ROI, region of interest. Scale bar, 15 μm. Values are means ± SE; n = 6–7. *P < 0.05 vs. Con.

Fig. 8. WD feeding increased total femoral artery TGF-β content. Immunohistochemistry was utilized to quantify total TGF-β content of femoral arteries following 16 wk of Con or WD feeding. Representative images on right were equally contrast enhanced for better print visualization. Scale bar, 20 μm. Values are means ± SE; n = 4–6. *P < 0.05 vs. Con.
Regional Arterial Stiffening and Dysfunction in Obesity

Vascular bed-specific effects of WD on arterial vasomotion. In addition to increased stiffness and remodeling, WD feeding induced differential vasomotor dysfunction in the femoral and coronary arteries. Specifically, femoral arteries exhibited endothelial vasodilator dysfunction, while coronary arteries exhibited a modest increase in vasoconstrictor responsiveness. Endothelial dysfunction, characterized by reduced dilation to endothelium-dependent (ACh) but not endothelium-independent (SNP) vasodilators, is a hallmark of obesity-related vascular dysfunction (24). Although not specifically examined in this study, it is likely that reduced NO bioavailability owing to increased oxidative stress contributes to WD-induced femoral vasodilator dysfunction. We have previously reported increased oxidative stress in this model of WD feeding (28, 33). Impaired endothelial function may underlie the structural remodeling of the femoral artery, since endothelium-derived substances (i.e., NO) modulate structural components of the vascular wall. For instance, a recent study demonstrated that increased aortic extracellular matrix cross-linking and stiffness involved endothelial dysfunction and activation of transglutaminase-2 that is typically inhibited by NO (48).

The lack of endothelial dysfunction in the coronary artery was somewhat surprising in light of previous evidence in similar models (2, 5, 17). Several factors may underlie this regional discrepancy in the development of obesity-related endothelial dysfunction. First, previous studies examined coronary microvascular endothelial function as opposed to our present analysis of the larger mouse coronary artery (2, 5, 17). In light of this, similarly to the lack of coronary remodeling/stiffening, these findings may suggest differential effects of WD feeding to induce vasodilator dysfunction in the coronary macrocirculation versus microcirculation. Second, local hemodynamics may contribute to reduced coronary vulnerability to dysfunction with WD feeding. We have previously demonstrated that the soleus muscle, but not the gastrocnemius muscle, feed artery exhibits resistance to obesity-related endothelial dysfunction (7). This protection likely relates to the relatively higher blood flows through this vessel compared with the gastrocnemius, since the soleus is recruited regularly during standing/walking whereas the gastrocnemius is recruited during running. Accordingly, access to a running wheel prevented gastrocnemius feed artery endothelial dysfunction (7). A similar resistance to dysfunction may be conferred to the coronary artery versus the femoral, owing to the high coronary flow necessitated by the constantly working heart.

Despite the lack of endothelial dysfunction, WD feeding induced a significant increase in coronary vasoconstrictor responsiveness to the thromboxane A₂ analog U46619. Vasocostriction to PE was not changed by WD feeding in the femoral artery. In the coronary artery, vasocostriction induced by exposure to 80 mM KCl was not enhanced, suggesting that the increased vasoconstrictor response to U46619 is a specific effect of WD as opposed to a generalized enhancement of vasocostructor pathways by WD feeding. Increased coronary vasocostriction to specific vasoconstrictor stimuli in obesity has previously been reported (6). Furthermore, this result is consistent with several previous reports. Specifically, increased prostanoid-mediated vasoconstriction and thromboxane receptor expression have been described in the carotid artery and aorta of mice with diet-induced obesity (45). In addition, enhanced prostaglandin/thromboxane receptor-mediated vasoconstriction attenuates skeletal muscle perfusion in the obese Zucker rat model (16). Thus, these data highlight a potential role of increased coronary vasoconstriction, in the absence of endothelial vasodilator dysfunction, as an important contributor to impaired coronary flow regulation in obesity and insulin resistance.

Conclusions. Taken together, our data reveal important regional variation in and novel structural alterations associated with the vascular pathology of premature vascular stiffening in vivo (i.e., PWV) associated with WD feeding. This is clinically relevant, since PWV is independently associated with cardiovascular disease risk (31). Our data reveal vasodilator dysfunction and remodeling in the femoral, but not coronary, artery and suggest an important link between femoral vasomotor dysfunction and structural remodeling in WD-fed mice. Future studies are necessary to fully delineate TGF-β involvement in femoral stiffening and novel femoral IEL fenestra remodeling as well as mechanisms underlying increased coronary vasoconstrictor responsiveness in WD-induced obesity.

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GRANTS

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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