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► **To cite this version:**

Aïcha Ben Zemzem, Xiaowen Liang, Laetitia Vanalderwiert, Camille Bour, Béatrice Romier-Crouzet, et al.. Early alterations of diabetic mouse aortas revealed by X-ray micro-CT. Synchrotron SOLEIL Highlights 2022, , pp.18-19, 2023. hal-04165366

HAL Id: hal-04165366

<https://hal.science/hal-04165366v1>

Submitted on 6 Aug 2023

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EARLY ALTERATIONS OF DIABETIC MOUSE AORTAS REVEALED BY X-RAY MICRO-CT

Diabetes is a major concern of our society that affects about 10% of the world population. Elastic fiber alterations due to diabetes increase the stiffness of large arteries through poorly understood structural alterations. To address this issue, we used synchrotron X-ray microtomography (μ -CT) with in-line phase contrast to image, in three dimensions, control and diabetic mice aorta with a voxel size of 650 nm and a field diameter and height of 1.3 mm. Image analysis shows that, in older diabetic mice, the elastic lamellae lose their internal scaffolds to become smoother. This suggests a link between the loss of their 3D lattice-like network and the reduction of their elasticity.

Diabetes is a chronic pathology affecting about 10% of the world population. It increases the probability of cardiovascular diseases and accelerates arterial stiffness. The effect of diabetes on the stiffness of the vascular system has been broadly estimated to correspond to 6–15 years of chronological aging [1]. This pathology is mainly characterized by an important level of blood glucose leading to the formation of cross-links within elastin fibers and collagens, resulting in a decrease of arterial wall distensibility. Large arteries such as the aorta contain, in their extracellular matrix, an amorphous elastic hydrophobic polymer named elastin, which is the major component of elastic fibers. Its entropic elasticity and its concentric lamellar organization within the aortic wall allow the aorta to remain in a reversible deformation domain during the cardiac cycle, which alternates phases of

overpressure and contraction. Alterations to elastin due to a pathological situation or aging permanently affect the arterial mechanics.

X-ray microtomography enables imaging of large sample volumes with sub-micrometric resolution associated to millimeter field size [2,3]. We have developed a protocol allowing us to harvest with a limited collapse the aorta from mice for X-ray μ -CT imaging.

EXPERIMENTAL RESULTS

Figure 1a shows a typical reconstructed raw 2D image plane extracted from a synchrotron X-ray μ -CT acquisition (2048³ voxels) at 0.65 μ m/voxel for an aortic segment of a 6-month control mouse. The sample was not sectioned and no labeling or contrasting agents were used. The phase-contrast image exhibits the different structures of the aortic environment from the thoracic region. Figure 1b shows an automatic segmentation of the elastic structures by a purpose-written fully automated program.

The control (C57Bl6J) and diabetic (db/db) mouse models share the same genetic background and therefore differences between the two groups would not originate from genetic variation between strains but solely from the pathological situation. The aspect of the elastic lamellae exhibits drastic differences (Fig. 2ab). The control presents a wavy aspect indicating that this elastic tissue still contains a *reserve length*. The reserve length of a spring, in addition to its stiffness, increases its resistance to stress under an acceptable constraint. This reserve length allows a spring to be stretched above its *a* i.e., its end-to-end distance. If a spring is stretched too much or altered, it loses its reserve length and thus its ability to be reversibly deformed again when overstretched.

One way to compare the wavy aspect to the smooth aspect is to compute, for a given segment, the ratio between its end-to-end

distance and its curvilinear length (i.e., its real length). In total, 127,180 measurements were made on control mice, 159,485 on diabetic mice. The box plot in Figure 3a reveals a smoother aspect for diabetic mice as compared to control animals ($p < 0.004$). Hence, the reserve length of the control group is significantly higher than that of diabetic mice, indicating an important modification of elastic lamellae in pathological mice. We have previously shown [3] that an inner lattice-like network is present inside the elastic structure, to which it seems to confer flexibility and resistance. 6-month-old diabetic mice exhibit a drastic destruction of this network in comparison of normal mice of the same age (Fig. 3b), suggesting a structural role for this lattice-like network.

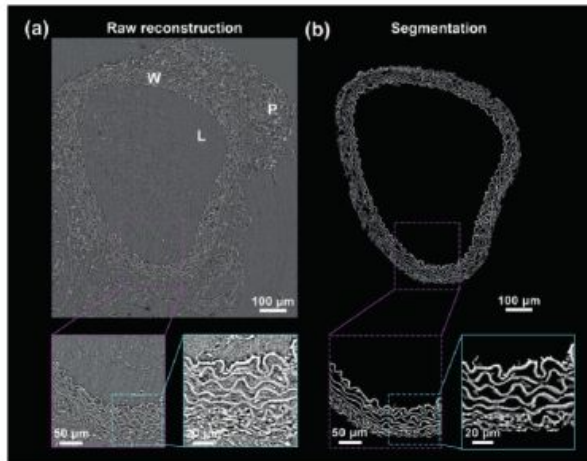
DISCUSSION

In-line phase-contrast synchrotron X-ray microtomography of the arterial wall with label-free tissues grants access to fine details of elastic structures. The strength of this method lies in its large field, its excellent resolution, and a good contrast for intramural elastic structures. Coupled to an automatic image analysis program, this technique provides information that cannot be obtained using other imaging methods. Moreover, for each sample, it allows the computation of thousands of measurements for a given feature, such as the ratio between Euclidean and geodesic distances of a segment of an elastic lamella.

Our results show that the reserve length of elastic lamellae is significantly reduced in diabetic mice together with the loss of filaments that support the wall. This suggests that elasticity is correlated to the existence of this lattice-like network that has disappeared at 6 months of diabetes.



Figure 1



ANATOMIX BEAMLINE



Associated publication

Early Alterations of Intra-Mural Elastic Lamellae Revealed by Synchrotron X-ray Micro-CT Exploration of Diabetic Aortas.



A. Ben Zemzem, X. Liang, L. Vanalderwiert, C. Bour, B. Romier-Crouzet, S. Blaise, M.J. Sherratt, T. Weitkamp, M. Dauchez, S. Baud, N. Passat, L. Debelle & S.Almagro.

International Journal of Molecular Sciences 23, 3250 (2022).



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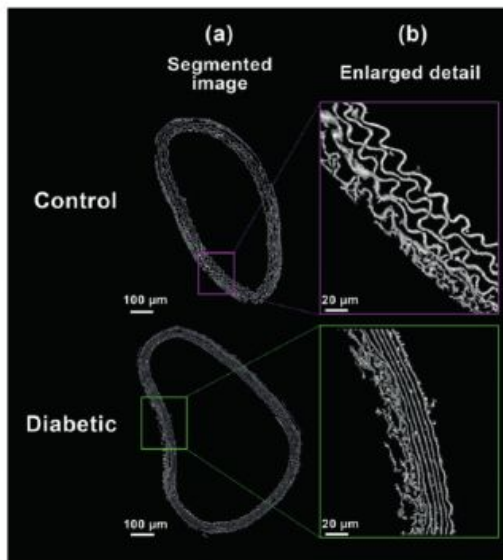
- [1] S.F. Vatner *et al.*, Front. Physiol. 12, 2068 (2021).
- [2] T. Weitkamp *et al.*, J. Phys. Conf. Ser. 2380, 012122 (2022).
- [3] A. Ben Zemzem *et al.*, FASEB J. 35, e21844 (2021).



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Figure 2



Captions

Figure 1: (a) Virtual slice obtained by in-line phase-contrast synchrotron microtomography of a paraffin-embedded 6-month control aortic segment. W, wall; P, perivascular tissue; L, lumen. (b) Segmentation of the elastic structures.

Figure 2: Effect of diabetes on the aortic structure of the mouse arterial wall. (a) Segmented images of control (upper row) and diabetic (lower row) mouse. (b) An enlarged view reveals smoother aspect for diabetic mouse than control mice.

Figure 3: (a) Box charts of end-to-end ratio obtained for control and diabetic 6-month mice ($p < 0.004$). (b) Lattice-like aortic filamentous networks imaged by synchrotron X-ray micro-CT. Diabetic mice lose this structure.

Figure 3

