Supplementary data file

Endothelial dysfunction and passive changes in the aorta and coronary arteries of diabetic db/db mice

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Methods and materials

Animals and preparation of samples

Male and female 8-week old db/db mice (C57BLKS/J-lepr\textsuperscript{db}/lepr\textsuperscript{db}) and age- and sex-matched db/db+ littermate controls (C57BLKS/J-lepr\textsuperscript{db/db+}) were purchased from Taconic Europe (Ry, Denmark). Supplementary table S1 show the number of animals used in the study. All animal care and experimental protocols in this study were conducted under the supervision of a veterinarian and in accordance with the Danish legislation of animal use for scientific procedures as described in the ‘Animal Testing Act’ (Consolidation Act No. 726 of 9 September 1993 as amended by Act No. 1081 of 20 December 1995) and approved by the Danish Animal Experiments Inspectorate (permission 2014-15-2934-01059).

Supplementary Table S1. Number of animals used for the different experiments described in the present study and weight and blood glucose.

| Experiment                      | Female         | Male           | Figure          |
|---------------------------------|----------------|----------------|-----------------|
|                                 | db/db+         | db/db          | db/db+          | db/db          |
| Echocardiography                | ND             | ND             | 7               | 7              |
| Aorta passive                   | 12             | 14             | 10              | 8              |
| Aorta myograph Passive/active   | 6              | 7              | 6               | 6              |
| Coronary passive                | 6              | 6              | 6               | 6              |
| Coronary function               | 6              | 6              | 6               | 6              |
| **Total number**                | **30**         | **33**         | **35**          | **33**         |
| Experimental weight             | 21 [10-27]     | 49 [39-53]*    | 29[18-36]       | 51[42-60]*     |
| Blood glucose                   | 7.4 [4.3;13.8] | 18[9.8;31.8]*  | 7.9[5.6;12.4]   | 28.5[17.2;33.3]* |

Data for weight and blood glucose are medians with 95% confidence interval measured at experimentation. *P<0.05, db/db versus respective db/+ control animals.
**Transthoracic echocardiography**

Echocardiography (Vevo2100, Visual Sonics, Toronto, Canada) was performed using a linear array probe (MS 550D, 22 – 55MHz) on the spontaneously breathing mouse that was placed in the left lateral decubitus position on a heating pad adjusted to 37 °C. The animal’s electrocardiography (ECG) signal was captured through copper electrodes on the heating pad.

**Passive mechanical studies in aorta segments**

Mechanical testing was performed on aorta segments. The ring specimens were pushed at minimal strain onto a tapered glass rod until the rod filled the lumen. These were then photographed twice using a Nikon microscope equipped with a circular polarization filter. Average wall thickness was estimated from traced (ImageJ 1.50e) cross-sectional areas at four random locations. An image of a millimeter scale was used for calibration (Supplementary Figure S1).

![Supplementary Figure S1. Representative image of aorta ring segment.](image)

The vessel wall area (outlined in yellow) was measured by calibrating it to a millimeter scale and the vessel wall thickness was derived by assuming a height of 1 mm for all segments.

**Passive properties of coronary arteries**
To determine the passive properties of the left anterior descending coronary artery (LAD) from db/db mice, arterial segments (lengths of 1.7 ± 0.5 mm) were mounted in an 110P pressure myograph system (Danish Myotechnology, Aarhus, Denmark) in a calcium-free PSS. The passive properties of the coronary arteries mounted in the pressure myograph were calculated as described in Supplementary Table S2.

**Supplementary Table S2.** Calculation of passive properties of coronary arteries mounted in a small vessel pressure myograph.

|   |   |   |
|---|---|---|
|   |   | Wall Thickness |
| **1-** | **WT** | Wall Thickness |
|   |   | $WT = \frac{(D_e - D_i)}{2}$ |
| **2-** | **W:L** | Wall:Lumen ratio |
|   |   | $W: L = \frac{(D_e - D_i)}{2D_i}$ |
| **3-** | **ID** | Incremental Distensibility |
|   |   | $ID = \frac{\Delta D}{D_i \Delta P} \times 100$ |
| **4-** | **σ** | Stiffness: Circumferential wall stress |
|   |   | Stress-strain relationship |
|   |   | $\sigma = \frac{(P \cdot D_i)}{2 \cdot WT}$ |
| **5-** | **ε** | Circumferential wall strain |
|   |   | $\varepsilon = \frac{(D_i - D_{i@10mmHg})}{D_{i@10mmHg}}$ |
| **6-** | **σ** | Stiffness |
|   |   | incremental elastic modulus , $E_{inc} = \frac{\delta \sigma}{\delta \varepsilon}$ |
|   |   | $\sigma = \sigma_{10mmHg} e^{\beta \varepsilon}$ |

De, external diameter; Di, internal diameter; ΔP, increase in pressure.
Results
Male and female diabetic (db/db) mice showed a significantly larger weight gain compared to the heterozygous (db/db+) littermates (Supplementary Figure S2A). The blood glucose levels were highly elevated in the diabetic db/db male and female mice compared to db/db+ control mice (supplementary Figure S2B).

Supplementary Figure S2. Body weight and blood glucose measured at different age in a subset of control (db/db+, N=11) and diabetic (db/db, N=14) male mice. Body weight is presented as means±s.e.mean. Blood glucose is presented as the median (IQR).

ECG measurements
The duration of the PR-interval, QT-interval and the corrected QT-interval on the ECG were significantly prolonged in diabetic animals (supplementary Figure S1).
Supplementary Figure S3. Representative electrocardiogram from a diabetic db/db mouse and a control db/db+ mouse. Note the M-shaped QRS complex.

**Transthoracic echocardiography**

Echocardiography of aorta showed decreased mean flow and maximal flow velocity in diabetic db/db mice (Supplementary Figure S4).
Supplementary Figure S4. Representative echocardiographic images of aorta in a db/db mouse. Representative images of the aortic arch (A, B-mode), the ascending aorta (B, M-mode) and flow through the aortic arch (C, pulse wave Doppler mode). Ao: aorta, Asc: ascending, d: diastole, Desc: descending, ID: inner diameter, Inn: innominate artery, L. C. Caro: left common carotid artery, L Sub: left subclavian artery, PA: right pulmonary artery.