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Worse cardiac remodeling in response to pressure overload in type 2 diabetes mellitus☆

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ABSTRACT

Background: Diabetic cardiomyopathy is characterized by cardiac structural and functional abnormalities. Additionally, chronic pressure overload conditions are highly prevalent amongst diabetic population and this association leads to a more severe myocardial impairment. The differences in myocardial pathophysiology between type 1 and type 2 diabetes mellitus (DM) still remain to be clarified. Thus, we aimed to investigate biventricular structural and functional changes promoted by the two types of DM and the impact of concomitant chronic pressure overload.

Methods: Wistar rats were injected with streptozotocin (Type 1 DM, T1DM) or fed with a hypercaloric diet (Type 2 DM, T2DM). Pressure overload was imposed in DM animals by aortic constriction and after 5 weeks of DM the cardiac function and structure were evaluated.

Results: Both types of DM promoted hypertrophy, increased fibrosis and advanced glycation end-products deposition, in the two ventricles. Interestingly, the induced myocardial alterations were distinct. While T1DM stimulated a pronounced hypertrophy and extracellular matrix remodeling, T2DM induced functional impairment. The negative impact of the association of DM with aortic constriction was more pronounced in T2DM, promoting impaired function and increased stiffness, particularly in the right ventricle.

Conclusions: Our study demonstrated that the two types of diabetes induce distinct cardiac alterations *per se* or when combined with chronic pressure overload. T1DM promoted a more extensive remodeling in cardiac structure while T2DM significantly impaired ventricular function. The impact of pressure overload was more notorious in T2DM as observed by worse myocardial remodeling, suggesting a higher susceptibility to the deleterious effects of chronic pressure overload, namely hypertension, among this diabetic population.

1. Background

Diabetes mellitus (DM) is a chronic metabolic disease that results from pancreas' inability to produce insulin, such as in Type 1 DM (T1DM), or from the incapacity of the organs to use insulin, as in Type 2 DM (T2DM). This impairment of insulin production and/or utilization and consequent hyperglycemia instigates multiple organ failure, including those of the cardiovascular system [1]. The incidence of diabetes mellitus has dramatically increased worldwide and in the next years the number of diabetic patients is expected to grow to epidemic proportions, particularly in developing countries, making it a major public health problem [2].

Despite the different triggering mechanisms, both T1DM and T2DM progress to diabetic cardiomyopathy, a condition characterized by myocardial structural and functional abnormalities in the absence of other comorbidities [3]. The role of cellular hypertrophy and extracellular matrix alterations for the development and progression of the myocardial remodeling has been described [4,5]. In fact, studies in humans [6–9] as well as in animal models [10,11] reported increased fibrosis and deposition of molecules involved in oxidative stress in both types of DM, resulting in increased myocardial stiffness. Furthermore, while T2DM impacts mostly diastolic function, T1DM has been described to mainly impair systolic function, even though we had previously described significant changes in the extracellular matrix responsible for the impaired distensibility upon stress conditions (β-adrenergic stimulation) in older T1DM rats. These initial changes in stiffness were aggravated when pressure-overload was superimposed as observed by the absence of inotropic and lusitropic response to β-adrenergic stimulation [11]. On

☆ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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the other hand, the idea that T2DM first affects the diastolic function was challenged by Ernande and colleagues [12]. This group reported that 28% of a T2DM cohort presented systolic alterations although with normal diastolic function and that longitudinal systolic strain was independently associated with diabetes.

Nonetheless, comparative studies about myocardial pathophysiology of these two conditions are lacking namely concerning: i) the ventricular mechanisms responsible for the different functional outcomes; ii) the exact onset of the first myocardial manifestations and iii) the impact of diabetes on the right ventricle (RV), which has been systematically ignored in most of the existing literature, despite its probable systemic actions [13].

Several chronic disorders are common amongst T1DM and T2DM patients, being hypertension the most prevalent, affecting 40–60% of diabetic population [2,14,15]. The concomitant presence of chronic pressure overload (CPO) and DM results in more profound myocardial hypertrophy and stiffness leading to a more severe cardiomyopathy [3,11,16,17].

Additionally, most of the previous studies in diabetic cardiomyopathy focus their attention only on the impact of DM in the left ventricle (LV), neglecting the RV [13]; since DM is a systemic condition, it is expected that the RV is also affected by the metabolic impairment.

In the present work we investigate the biventricular myocardial structural and functional changes promoted by T1DM and T2DM and the impact of CPO imposed on these conditions.

2. Methods

2.1. Experimental animal model

This study was made according to the Guide for the Care and Use of Laboratory Animals published by the NIH (NIH Publication no. 85-23, revised 2011) and with the Portuguese law of animal welfare (DL 129/92, DL 197/96; P 1131/97). The Faculty of Medicine of the Universidade do Porto is a governmental institution granted with approval to perform animal experiments by the Portuguese Government.

Male Wistar Han rats were obtained from Charles River (Spain) and housed in groups of 5 *per* cage in a room at 22 °C with a controlled environment under a 12:12-h light–dark cycle and with unlimited access to food and water.

2.1.1. Chronic pressure overload

Left ventricular CPO was induced by aortic constriction in rats with 5 weeks of age (n = 20). The animals were anesthetized with sevoflurane (8% for induction and 2.5–3% for maintenance), the aorta was exposed and a blunted 23-gauge needle (0.64 mm diameter) was placed parallel to the aorta. A ligature (5-0 silk; PROLENE) was firmly tied around both the aorta and the needle. The needle was then removed, leaving the internal diameter of the aorta approximately equal to the needle.

2.1.2. Induction of diabetes mellitus

Two weeks later, 7 week-old rats were randomly divided and injected with streptozotocin (STZ; 65 mg/kg, ip; DM1 group, n = 8) or the same volume of vehicle (citrate buffer 5 mL/kg, pH = 4.5; CT1 group, n = 9). Half of the banded animals also received STZ resulting in an additional group (DB1, n = 7).

To induce T2DM, 7 week-old rats were randomly divided and fed with a hypercaloric diet rich in lipids, carbohydrates and increase salt content (5.4 kcal/g, F2685, BioServe, DM2 group, n = 10) or a regular diet (2.9 kcal/g, A04; Scientific Animal Food & Engineering; CT2 group, n = 10). Half of the banded animals were also fed with this hypercaloric diet resulting in an additional group (DB2, n = 13). The detailed diet composition is described in Table 1.

Table 1
Diet composition.

	Regular diet (AO4)	Hypercaloric diet (F2685)
Calorie composition (kcal/g)	2.9	5.4
Protein (%)	16.1	20.5
Carbohydrate (%)	3.2	34.7
Monosaccharides (gm/kg)	17.3	1
Disaccharides (gm/kg)	6.8	182
Polysaccharides (gm/kg)	26.1	157
Fat (%)	3.1	36.0
Saturated fat (gm/kg)	6.5	141
Monounsaturated fat (gm/kg)	20.0	162
Polyunsaturated fat (gm/kg)	1.2	40.2
Sodium (%)	0.25	0.4

The presence of DM was confirmed by plasma glycemia > 250 mg/dL (Breeze, Bayer). Five weeks later cardiac structure and function were evaluated and samples collected.

The experimental groups resultant from this protocol design are described in Table 2.

2.2. Echocardiogaphic assessment

Echocardiographic evaluation was performed using a 10 MHz transducer (GE Vivid 7). Animals were anesthetized (ketamine:xylazine, 75:5 mg/kg, ip) and allowed to stabilize for 15 min. From the left parasternal short-axis view, two-dimensional guided M-mode tracings were made just below the mitral valve at the level of the papillary muscles for measurements of the interventricular septum (IVS, mm), posterior wall thickness (LVPW, mm) and left ventricular diameter (LVD, mm) during systole and diastole. Left ventricular mass (LVM, mg) was calculated by the formula $= 1.04 \times (\mathrm{IVSd} + \mathrm{LVDd} + \mathrm{LVPWd})^3 - \mathrm{LVD}^3$. All these parameters were normalized to body surface area (IVSI, LVPWI, LVDI and LVMI) as described previously [11]. Three representative cycles were measured *per* rat and their average was calculated.

2.3. Hemodynamic evaluation

The animals were anesthetized with sevoflurane (8% for induction and 2.5–3% for maintenance), intubated for mechanical ventilation (Dual Mode, Kent Scientific), and placed over a heating pad (body temperature was maintained at 37 °C). To compensate for the perioperative losses, the right jugular vein was cannulated for fluid administration (prewarmed 0.9% NaCl solution, 10 mL/kg/h). A median sternotomy was made and the aorta dissected for record flux with an ultrasonic flow probe, connected to a flowmeter (Transonic Systems Ithaca). High-fidelity tip pressure–volume catheters were inserted into the LV and RV (SPR-847, 2F and PVR-1045, 1F respectively; Millar Instruments). After complete instrumentation, the animal was allowed to stabilize for 15 min. Data was continually acquired digitally (MPVS 300, Millar Instruments), recorded at 1000 Hz (ML880 PowerLab 16/30, Millar instruments), and analyzed off-line by software PVAN 3.5 (Millar Instruments).

Hemodynamic recordings were made under basal conditions with respiration suspended at end-expiration. The hemodynamic parameters analyzed were the peak systolic pressure (P_{Syst}), maximum rate of pressure rise (dP/dt_{max}), end-diastolic pressure (EDP) and time constant of isovolumetric relaxation (Tau). Ascending aorta occlusions were performed during the diastole separating two heartbeats. The first beat was control, and the second beat was test heartbeat. The contractile reserve was then calculated as the % of increase of the systolic pressure according to the formula: [(P_{Syst} of the second beat − P_{Syst} of the first beat) / P_{Syst} of the first beat)] × 100. This intervention evaluates the effect of increased afterload without changes of preload or long-term load history. Conductance Calibration: Parallel conductance values were obtained by the injection of approximately 100 μL of 10% NaCl into