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The usefulness of short-term high-fat/high salt diet as a model of metabolic syndrome in mice

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ABSTRACT

Diabetic cardiomyopathy (DC) describes diabetes-associated changes in the structure and function of myocardium that are not directly linked to other factors such as hypertension. Currently there are some models of DC; however, they take a large time period to mimic key features. In the present study, we investigated the effects of a short-term high-fat/high salt diet (HFHS) treatment on myocardial function and structure, and vascular reactivity in C57BL/6 male mice. After 14 weeks HFHS induced hypertension (MAP = 144.95 ± 16.13 vs 92.90 ± 18.95 mm Hg), low glucose tolerance (AUC = 1049.01 ± 74.79 vs 710.50 ± 52.57 a.u.), decreased insulin sensitivity (AUC = 429.83 ± 35.22 vs 313.67 ± 19.55 a.u.) and increased adiposity (epididymal fat weight 0.96 ± 0.10 vs 0.59 ± 0.06 OW/BW × 10^2), aspects present in metabolic syndrome. Cardiac evaluation showed diastolic dysfunction (E/A ratio = 1.20 vs 1.90 u.a.) and cardiomyocyte hypertrophy (cardiomyocyte area = 502.82 ± 31.46 vs 385.58 ± 22.11 μm^2). Lastly, vascular reactivity was impaired with higher contractile response (136.10 ± 3.49 vs 120.37 ± 5.43%) and lower response to endothelium-dependent vasorelaxation (74.01 ± 4.35 vs 104.84 ± 3.57%). In addition, the diet was able to induce an inward coronary remodeling (vascular total area: SCNS 6185 ± 800.6 vs HFHS 4085 ± 213.7 μm^2). Therefore, we conclude that HFHS short-term treatment was able to induce metabolic syndrome-like state, cardiomyopathy and vascular injury working as an important tool to study cardiometabolic diseases.

1. Introduction

Diabetes mellitus (DM) reduces life expectancy as a major cause of complications such as renal failure, ischemia and myocardial infarction [1]. Some important studies reveal that vascular complications of DM are associated with multiple risk factors, such as obesity, hyperlipidemia, hyperglycemia and hypertension, parameters found in the metabolic syndrome [1–3].

Diabetic cardiomyopathy (DCM) is recognized by impaired myocardial relaxation, left ventricular stiffening, progressive development of interstitial fibrosis and cardiomyocyte hypertrophy. Although diabetes experimental animal models are extensively used in the literature and diabetic cardiomyopathy is known as a progressive disease that begins early in the diabetes onset, the natural history and progression of DCM has never been directly studied [4].

It has been showed in a few studies that a diet rich in macronutrients such as lipids and carbohydrates contribute to cardiometabolic alterations, via increased adiposity, cholesterol and glycemic

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levels [5]. However, these diets do not induce representative alterations of cardiovascular dysfunctions as verified in cardiomyopathy [6]. Yu and cols [7], tested different diets with high-salt content and observed dysfunctions in the ventricular dynamics, hindering blood ejection force, which is common in cardiomyopathy [8]. However, the long-term treatment required in the presented models, which varies from 20 to 30 weeks, represents an important limitation.

In this perspective, we aimed to evaluate the cardiovascular damage of a high-fat/high-salt diet as well as the metabolic profile in mice.

2. Methods

2.1. Animals and protocols

Twenty male C57BL/6 mice (four-week-old) from the Federal University of the Minas Gerais (Belo Horizonte, Minas Gerais, Brazil) were kept under standard conditions (12 h light/dark cycles, 21 ± 2 °C) and with free access to chow and water. All procedures complied with both the standards stated in the Guide for the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and the Care and Use of Laboratory als (Institute of Laboratory Animal Resources, National Academy of Sciences, Bethesda, Md, 1996) and were conducted under conditions approved by the local animal ethics committee, CETEA/UFMG.

Mice were randomly divided into two groups: standard chow/normal salt diet (NCNS), composed of 66.0% carbohydrate, 23.0% protein, 11.0% fat and 0.2% NaCl, presenting a total of 9.13 kJ/g (Labina-Purina®, Brazil). High-fat chow/high-salt diet (HFHS) was composed of 26.0% carbohydrate, 14.0% protein, 60.0% fat (50% from lard and 10% from soybean oil) and 7.25% NaCl, presenting a total of 23.38 kJ/g. Both groups were fed respective experimental diets for 14 weeks.

2.2. Measurements of body weight, food intake and tissue collection

We measured body weight (BW) and food intake once and twice, respectively, each week during the treatment. After 14 weeks of treatment mice were killed by decapitation and samples of heart; epididymal, retroperitoneal and mesenteric white adipose tissue were collected and weighed to determine the organ weight (OW). Data was expressed as $OW/BW \times 10^2$.

2.3. Glucose tolerance and insulin sensitivity tests

The intraperitoneal glucose tolerance tests (IPGTT) and intraperitoneal insulin tolerance test (IPITT) were performed in all animals of each group as described previously [37]. Briefly, D-glucose (2 mg/g body weight, ip) was injected into overnight fasted mice. Glucose levels from tail blood samples were monitored at 0, 15, 30, 60, and 90 min after injection using an Accu-Check glucometer (Roche-Diagnostics®, Indianapolis, USA). Insulin sensitivity test was performed on overnight-fed mice, after intraperitoneal injection of insulin (0.75 units/kg body weight; Sigma–Aldrich®, St. Louis, USA). Tail blood samples were taken at times 0, 30, 60, 90 and 120 min after injection for measurement of blood glucose levels. Both tests were performed in the last week of treatment.

2.4. Blood pressure measurements

Arterial blood pressure was measured in conscious mice at week 13 using a computerized and non-invasive tail-cuff system (CODAtm system, Kent Scientific) following the original protocol described by Daugherty [38]. In order to habituate the animals to the device and reduce variations in response to stress, arterial blood pressure measurements were carried out daily for one week just prior to the experiment. The first five cycles were discarded and the average of the 15 subsequent arterial blood pressure measurement cycles was used. We performed arterial blood pressure measurements between 2:00–5:00 pm [38].

2.5. Isometric tension measurement

We obtained rings (≈2 mm) from the descending thoracic aorta, free of adipose and connective tissue from mice immediately after sacrifice. We placed these rings in Krebs-Henseleit solution with the following composition (mM/L): NaCl 110.8, KCl 5.9, $NaHCO_3$ 25.0, $MgSO_4$ 1.07, $CaCl_2$ 2.49, NaH_2PO_4 2.33, and glucose 11.51, at 37 °C, pH of 7.4, under a tension of 0.5 g, for 1 h for equilibration. The presence of a functional endothelium was assessed by the ability of acetylcholine (ACh) (10^{-5} M; Sigma-Aldrich®) to induce > 70% relaxation of vessels precontracted with phenylephrine (Phe) (10^{-5} M; Sigma-Aldrich®). In all experiments, the aortic rings were exposed twice to depolarizing Krebs-Henseleit solution (K^+ 60 mM). After washout, the contractile responses to Phe or relaxation responses to ACh and sodium nitroprusside (SNP) were recorded. A concentration-response curve to Phe was recorded as percentage of the maximum contraction obtained following tissue stimulation with high K^+ using half-log concentration increments (10^{-9}–10^{-5} M). Increasing concentrations of Ach (10^{-10}–10^{-5} M) or SNP (10^{-11}–10^{-6} M) were administered at half-log increments to evaluate endothelium dependent and independent vasorelaxation, respectively. A selective cyclo-oxygenase inhibitor, indomethacin (10^{-6} M), was added to the Krebs' solution 30 min before the construction of a concentration–response curve. The drug-induced responses were measured using the MacLab Chart v 7.2.1 program (AD Instruments, Australia).

2.6. Echocardiographic analysis

Transthoracic echocardiographic examination was performed in mice using a high-frequency, high-resolution echocardiographic system consisting of a VEVO 2100 ultrasound machine equipped with a 30–40 MHz bifrequencial transducer (Visual Sonics, Toronto, Canada). Anesthesia was induced in mice with 5% isoflurane and maintained via a nose cone with 1.25% isoflurane. The anterior chest was shaved and the mice were placed in supine position on an imaging stage equipped with built-in electrocardiographic electrodes for continuous heart rate monitoring and a heater to maintain body temperature at 37 °C. High-resolution images were obtained in the right and left parasternal long and short axes and apical orientations. Standard B-mode images of the heart and pulsed Doppler images of the mitral and tricuspid inflow were acquired. Left ventricular dimensions and wall thickness were measured at the level of the papillary muscles in left and right parasternal short axis during the end systole and end diastole. Left ventricular ejection fraction, fractional shortening, and mass were measured. All the measurements and calculations were done in accordance with the American Society of Echocardiography. The following M-mode measurements were performed: ventricular internal dimensions at diastole (LVIDD), left ventricular internal dimensions at systole (LVIDS), left ventricular posterior wall dimensions at diastole (LVPWD), left ventricular posterior wall dimensions at systole (LVPWS), interventricular septal dimensions at diastole (IVSDD) and interventricular septal dimensions at systole (IVSDS). Based on these parameters the diastolic left ventricular volumes (EDLVV), systolic left ventricular volumes (ESLVV), fractional shortening (FS), left ventricular ejection fraction (EF), stroke volume (SV), and cardiac output (CO) were calculated. Also, the radial strain from the bidimensional long axis view of the left ventricle was performed using the Vevostrain software. The following parameters were evaluated: velocity, displacement, strain, and strain rate the E/A ratio was derived using the pulse wave Doppler recording of mitral valve leaflet tips provides mitral inflow velocity patterns from which early diastolic velocity (E), late diastolic velocity with diastolic