

# The effects of a high-fat diet on left ventricular fibrosis

Katarzyna Czarzasta<sup>1</sup>, Łukasz Koperski<sup>2</sup>, Łukasz Fus<sup>2</sup>, Olena Wojno<sup>1</sup>,  
Barbara Górnicka<sup>2</sup>, Agnieszka Cudnoch-Jędrzejewska<sup>1</sup>

<sup>1</sup>Department of Experimental and Clinical Physiology, Laboratory of Centre for Preclinical Research, Medical University of Warsaw, Warsaw, Poland

<sup>2</sup>Department of Pathology, Medical University of Warsaw, Warsaw, Poland

## INTRODUCTION

Obesity is one of the major risk factors for cardiovascular diseases [1]. Experimental and clinical studies have shown that left ventricular (LV) hypertrophy and altered extracellular matrix in the myocardium occur in obesity, and they both significantly increase myocardial stiffness [2]. The incidence of diastolic dysfunction increases with the severity of obesity. Probably, diastolic dysfunction is secondary to the cardiotoxicity of free fatty acids (FFA) [3]. The main cause of increased FFA in cardiomyocytes is a diet rich in saturated fatty acids (SFA) [4].

The occurrence of changes in LV morphology has been reported in numerous studies on genetic models of obesity. However, these modifications are still poorly understood in animal models of obesity induced by a high-fat diet (HFD). Therefore, the aim of the study was to investigate the effect of a HFD (followed for 12 or 16 weeks) on LV fibrosis in Sprague Dawley (SPRD/Mol/Lod) rats.

## METHODS

### *Animals*

The studies were conducted on 23 male SPRD rats. All experimental procedures were approved by the Local Animal Research Ethics Committee (33/2011; release date: 29.11.2011). Four-week-old rats were put on a HFD (containing 31% fat, 17.1% protein, 35.5% carbohydrates, 0.18% sodium, 3842 kcal/kg; Labofeed B, Kcynia, Poland) for 12 weeks (16-week-old rats; HFD 16-wk; n = 5) or for 16 weeks (20-week-old rats; HFD 20-wk; n = 6). The control group for rats on a HFD comprised rats fed a normal-fat diet (NFD; containing 3.6% fat, 17.4% protein, 60% carbohydrates, 0.2% sodium, 2864 kcal/kg; Labofeed B, Kcynia, Poland) for 12 weeks (16-week-old rats; NFD 16-wk; n = 6) or for 16 weeks (20-week-old rats; NFD 20-wk; n = 6).

### *Heart harvesting*

At the end of the experiment the LV of each heart was removed for histopathological analysis. The LV was fixed in a 4% formaldehyde solution.

### *Histopathological analysis*

Fixed LVs were embedded in paraffin, cut into 3- $\mu$ m sections, and stained routinely with haematoxylin and eosin for morphological examination as well as with a ready-to-use Masson's Trichrome staining kit (Masson's Trichrome Stain Kit; Polysciences, Inc., Warrington, PA, USA). The amount of fibrosis of the LV was assessed by measuring the total cardiomyocyte cross-sectional area (stained red with Masson's Trichrome stain) and the total area of fibrosis (stained blue with Masson's Trichrome stain) (Fig. 1). Measurements were performed on 9–12 representative optical fields for each heart. The sections were viewed at 200  $\times$  magnification on light microscopy (Nikon Eclipse 80i) with a digital colour camera (QImaging QICAM Fast 1394). The total cross-sectional area of cardiomyocytes and the total area of fibrosis were assessed semi-automatically using Image-Pro Plus 7.0 software (Media Cybernetics, Rockville, MD, USA), expressed in  $\mu$ m<sup>2</sup> and as a percentage of the analysed visual field area.

### *Statistical analysis*

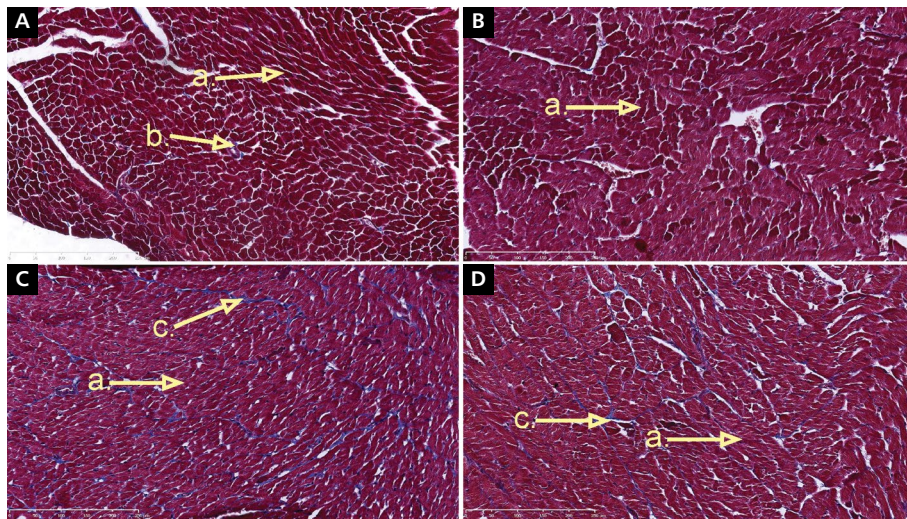
Statistical analysis was performed using Statistica software (version 10; Dell, Round Rock, TX, USA). Parametric tests (One-way ANOVA and Multi-way ANOVA) were used for comparison of the mean values with normal distribution of each characteristic. Nonparametric tests (Kruskal-Wallis and Mann-Whitney tests) were used for variables with non-normal distribution. The differences were considered statistically significant if  $p < 0.05$ . All values presented are expressed as mean  $\pm$  standard error of the mean (SE).

#### Address for correspondence:

Łukasz Koperski, MD, Department of Pathology, Medical University of Warsaw, ul. Pawińskiego 7, 02–106 Warszawa, Poland, e-mail: lkoperski@wum.edu.pl

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**Figure 1.** A. Left ventricular fibrosis area in the normal-fat diet (NFD) 16-week rats. NFD 16-week — rats maintained on a normal-fat diet for 12 weeks; a. — cardiomyocytes; b. — perivascular fibrosis; B. Left ventricular fibrosis area in the NFD 20-week rats. NFD 20-week — rats maintained on a normal-fat diet for 16 weeks; abbreviations as in panel A; C. Left ventricular fibrosis area in the high-fat diet (HFD) 16-week rats. HFD 16-week — rats maintained on a high-fat diet for 12 weeks; c. — fibrosis in the extravascular matrix of the left ventricle; other abbreviations as in panel A; D. Left ventricular fibrosis area in the HFD 20-week rats. HFD 20-week — rats maintained on a high fat diet for 16 weeks; abbreviations as in panel C

## RESULTS

### *Characteristics of the animals*

Significant differences were found between the body weights of the NFD 20-wk rats and the NFD 16-wk rats ( $381 \pm 8.52$  g vs.  $317 \pm 3.87$  g,  $p < 0.001$ ) and between the HFD 20-wk rats and the HFD 16-wk rats ( $388 \pm 9.80$  g vs.  $317 \pm 2.30$  g,  $p < 0.001$ ).

### *Effect of a HFD on LV fibrosis*

The LV fibrosis area in relation to the entire LV area was significantly larger in the HFD 16-wk rats than in the NFD 16-wk rats ( $8.29\% \pm 0.80\%$  vs.  $2.76\% \pm 0.19\%$ ,  $p < 0.05$ ), and it was also larger in the HFD 20-wk rats than in the NFD 20-wk rats ( $8.79\% \pm 0.49\%$  vs.  $2.84\% \pm 0.2\%$ ,  $p < 0.05$ ).

However, the cross-sectional area of the LV cardiomyocytes in relation to the entire LV area was significantly smaller in the HFD 16-wk rats in comparison with the NFD 16-wk rats ( $85.06\% \pm 6.75\%$  vs.  $97.24\% \pm 0.19\%$ ,  $p < 0.05$ ), and it was smaller in the HFD 20-wk rats than in the NFD 20-wk rats ( $91.21\% \pm 0.49\%$  vs.  $97.16\% \pm 0.23\%$ ,  $p < 0.05$ ).

## DISCUSSION

In this study, we presented the LV fibrosis process on an animal model of obesity induced by HFD, which is more similar to the development of obesity in humans than genetic models of obesity. The LV fibrosis area in relation to the entire LV area was significantly larger in the HFD 16-wk rats and the HFD 20-wk rats compared with the NFD 16-wk rats and the NFD 20-wk rats. The above results confirm previous experimental

research conducted on genetic rodent models of obesity [5]. It was shown that hyperlipidaemia, which accompanies obesity, can induce a systemic inflammatory state that activates fibroblasts in the myocardium, stimulating them to increase the production of collagen and leading to interstitial fibrosis [6]. Interstitial fibrosis of the myocardium was also observed in our research. Similarly, studies conducted on Zucker rats showed elevated levels of collagen types I and III in the LV [7]. However, the studies of Silva et al. [8] conducted on Wistar rats showed that a HFD resulted in increased levels of collagen type I in the LV, whereas the expression of collagen type III did not change.

Furthermore, it has been demonstrated that in obese people factors involved in the synthesis of myocardial collagen, such as procollagen type III, can correlate positively with LV diastolic dysfunction [9].

Based on the available literature, it can be assumed that the direct cause of the inflammatory response that activates fibroblasts is an increase in the levels of fatty acids in cardiomyocytes. Fatty acids stimulate  $\beta$ -oxidation in the mitochondria, leading not only to increased energy production in the form of adenosine triphosphate but also to the reactive oxygen species level, which in turn leads to cardiomyocyte apoptosis [10]. The present study reported a decrease in the cross-sectional area of LV cardiomyocytes in relation to the entire LV area in HFD 16-wk and HFD 20-wk rats in comparison with NFD 16-wk and NFD 20-wk rats, respectively. The increased fatty acid levels in the myocardium were probably due to the HFD, but not the time of its administration.

A HFD rich in SFA caused an increase in LV fibrosis in SPRD rats. Better knowledge of cellular mechanisms leading to LV fibrosis will contribute to the development of a pharmacological treatment of cardiovascular diseases induced by obesity.

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**Conflict of interest:** none declared

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