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The influence of high fat diet on gut dysbiosis and myocardial function

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INTRODUCTION

Obesity is a chronic disease which is considered to be a global pandemic according to the World Health Organization [1]. Experimental and clinical studies have shown that obesity caused by a high fat diet (HFD) is associated with the occurrence of myocardial fibrosis, atrial fibrillation, and left ventricular (LV) diastolic dysfunction (LVDD) [2, 3]. Recently, the influence of gut microbiota on the human body as well as on the activity of the cardiovascular system has been highlighted [4]. Obesity and a HFD lead to gut dysbiosis, which is defined as an imbalance of the composition of the microbiota. The occurrence of gut dysbiosis correlates with chronic systemic inflammation and consequently with an increased risk of cardiovascular diseases [5]. Gut dysbiosis caused by a HFD results in an increased permeability of the gut epithelium and higher lipopolysaccharide (LPS) translocation into circulation, which contributes to metabolic endotoxemia [2]. LPS activates toll-like receptors (TLRs, such as TLR4 and TLR6), which in turn prompts the synthesis of various proinflammatory cytokines

[6]. As a result, low-intensity systemic inflammation is induced and contributes to the onset and development of cardiovascular diseases [3, 5]. However, the mechanism of the influence of gut dysbiosis casued by a HFD on myocardial function remains to be elucidated. Therefore, the aim of the study was to investigate whether myocardial dysfunction under conditions of a HFD are related to gut dysbiosis.

METHODS

Animals

The study was conducted on 14 male Sprague Dawley rats (SPRD/Clzd). At four weeks of age, the rats were divided into two groups: rats on a normal fat diet (NFD, n = 7, containing: 3.6% fat, 17.4% protein, 60% carbohydrates, 0.2% sodium, 2864 kcal/kg; Labofeed B, Kcynia, Poland), mean body weight [b.w.]: 335 g; and rats on a high fat diet (HFD, n = 7; containing: 31% fat, 17.1% protein, 35.5% carbohydrates, 0.18% sodium, 3842 kcal/kg; Labofeed B, Kcynia, Poland), mean b.w.: 332 g.

The study was approwed by the Local Animal Research Ethics Committee in Warsaw (226/2016).

Then the rats were exposed to the following procedures:

Fecal microbiota analysis

Faeces for microbiota analysis were collected from randomly selected 12-week old rats on a NFD (n = 3; mean b.w.: 340 g) and on a HFD (n = 3; mean b.w.: 348 g). A fecal microbiota analysis was carried out at a veterinary laboratory (ALAB Weterynaria, Warsaw, Poland). The quantitative evaluation of fecal bacteria was performed using the serial dilution method. 0.1ml of the given dilution was placed in duplicate on agar plates, which were incubated for 48 hours in aerobic or anaerobic conditions at a temperature of 37°C.

Echocardiography analysis

The same rats on a NFD (n = 3) and a HFD (n = 3) from which faeces were collected for microbiological analysis were subjected to the echocardiography study. A baseline echocardiography was performed under general anesthesia (Ketamine 10 mg/100 g b.w., intraperitoneal with Xylazine 1 mg/100 g b.w., intraperitoneal) and a Vivid i machine (Biosense Webster Inc.) equipped with a 12 MHz electronic transducer was used. The parameters related to the systolic and diastolic functions of the LV, the mitral annular plane

systolic excursion, the tricuspid annular plane systolic excursion, the LV outflow tract velocity time integral, and the heart rate were analyzed.

Immediately after the echocardiography test, all of the rats on a NFD (n = 7) and on a HFD (n = 7) were euthanized to enable plasma and LV tissue collection for following biochemical analysis:

Real Time PCR (RT-PCR) analysis

Multiplex RT-PCR analysis was conducted in accordance with the Applied Biosystems protocol using the TaqMan® RNA-to-Ct™ 1-Step Kit and a primer for the rat TLR4 receptor (Rn00569848_m1; Applied Biosystems), a primer for the rat TLR6 receptor (Rn02121288_s1; Applied Biosystems) and a housekeeping gene primer for the rat GADPH (Rn01775763_g1).

Western Blot analysis

Western Blot analysis was performed according to the protocol described earlier [7].We used the following antibodies: a primary mouse monoclonal antibody against TLR4 (1:200 dilution, sc-293072; Santa Cruz Biotechnology, Dallas, TX, USA), a primary mouse monoclonal antibody against TLR6 (1:1000 dilution, ab228424; Abcam, Cambridge, UK), a primary mouse monoclonal antibody anti-β Actin (1:1000 dilution, ab8226; Abcam), a secondary antibody: goat anti-mouse conjugated to Horseradish Peroxidase (HRP) (1:2000 dilution, ab205719; Abcam). TTBS solution (0.5% Tween 20 in TBS; Bio-Rad, Hercules, CA, USA) containing 5% skim milk was used as a blocking factor. TLR4 and TLR6 protein levels were standardized by β-actin.

ELISA analysis

Plasma LPS concentrations were determined by enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instructions (Rat Lipopolysaccharides [LPS] ELISA Kit; MBS268498; MyBioSource Inc., San Diego, CA, USA)

Statistical analysis

Statistical analysis was performed using the Statistica software version 13. The obtained data were analysed with the Student's t-test. The differences were considered statistically significant if $P < 0.05$. The results were reported as the mean and the standard errors.

RESULTS

Gut microbiota composition

In the fecal cultures of the NFD rats as well as of the HFD rats were observed (measured as colony-forming units per gram — cfu/g) in the phylum *Proteobacteria: Escherichia coli* (NFD = 2×10^7 [1×10^7] cfu/g vs HFD = 1×10^7 [3×10^6] cfu/g); phylum *Firmicutes: Enterococcus spp*. (NFD = 7×10^7 [4×10^7] cfu/g vs HFD = 2×10^7 [6×10^6] cfu/g), *Lactobacillus spp*. (NFD = 9×10^9 [1×10^9] cfu/g vs HFD = 1×10^9 [3×10^8] cfu/g), *Clostridium spp*. (NFD = 7×10^4 [1×10^3] cfu/g vs HFD = 6×10^3 [1×10^3] cfu/g); phylum *Bacteroidetes: Bacteroides spp.* (NFD = 3×10^6 [1×10^6] cfu/g vs HFD = 5×10^6 [3×10^6] cfu/g); phylum *Actinobacteria*: *Bifidobacterium spp.* (NFD = 1×10^9 [3×10^8] cfu/g vs HFD = 1×10^9 [3×10^8] cfu/g). However, there were no significant differences in the fecal cultures composition between NFD and HFD.

Echocardiography analysis

The echocardiography analysis was shown mild left atrium (LA) hypertrophy in the HFD rats (5.69 [0.22] mm) compared to the NFD rats (4.63 [0.11] mm; P <0.05). There was also a decrease in the è-wave velocity in the HFD rats (0.05 [0.003] m/s) in comparison with the NFD rats (0.06 [0.001] m/s; P <0.05), which may indicate LVDD. Nevertheless, the other echocardiographic parameters, including LV systolic function, did not differ between the HFD rats and the NFD rats.

mRNA expresion and protein levels of TLR4 and TLR6 mRNA in the left ventricular

TLR4 (P <0.001; Figure 1A) and TLR 6 (P <0.001; Figure 1B) mRNA expression as well as TLR4 protein level (P <0.001; Figure 1C) were higher in the LV in the HFD rats compared with the NFD rats. Whereas there were no differences in the TLR6 protein level in the LV between both groups of rats (P = 0.87; Figure 1D).

Plasma LPS level

There were no differences in the plasma LPS level between the NFD rats (6.98[0.67] ng/ml) and the HFD rats (9.04[0.82] ng/ml; p=0.078).

DISCUSSION

The main observation emerging from the present study is that HFD may contribute to the development of LA hypertrophy and LVDD via TLRs.

Microbiological analysis showed lack of differences in fecal microbiota composition between rats on HFD and NFD. However, available researches showed that in obese patients were observed the reduction in the number of bacteria from the phylum *Firmicutes* and an increase in the number of bacteria from the phylum *Bacteroidetes* [8]. It was also shown that LPS is one of the metabolites of bacteria of the phylum *Bacteroidetes* [6]. The primary point of LPS uptake is TLR receptors [6]. In the present study, an increase in the expression of TLR4 and TLR6 mRNA nad TLR4 protein level in the LV of HFD rats was demonstrated. The results of previous studies confirm the association of excessive fat consumption and obesity with the stimulation of TLR receptors, inducing low-grade generalized chronic inflammation, which increases cardiovascular risk [9]. In our previous study, we have shown a link between the development of systemic inflammation accompanying obesity and an increase in myocardial fibrosis, which may affect the heart function [3]. Similarly, in this study, we have demonstrated that HFD contributes to mild LA hypertrophy and the development of LVDD. Clinical studies were shown that obese patients were more likely to have an enlarged LA diameter, which was strongly associated with atrial fibrillation [10]. Moreover, it was found that a high body mass index is an independent risk factor for the development of atrial fibrillation [10].

In conclusion, the present study indicates that myocardial dysfunction in rats on a HFD may be related to TLR receptors.

Study limitations:

Evaluation of the fecal microbiota composition and echocardiography was performed on a small number of rats (NFD = 3, HFD = 3), which affected statistical power of the study. The fecal microbiota composition was assessed using a traditional microbiological method.

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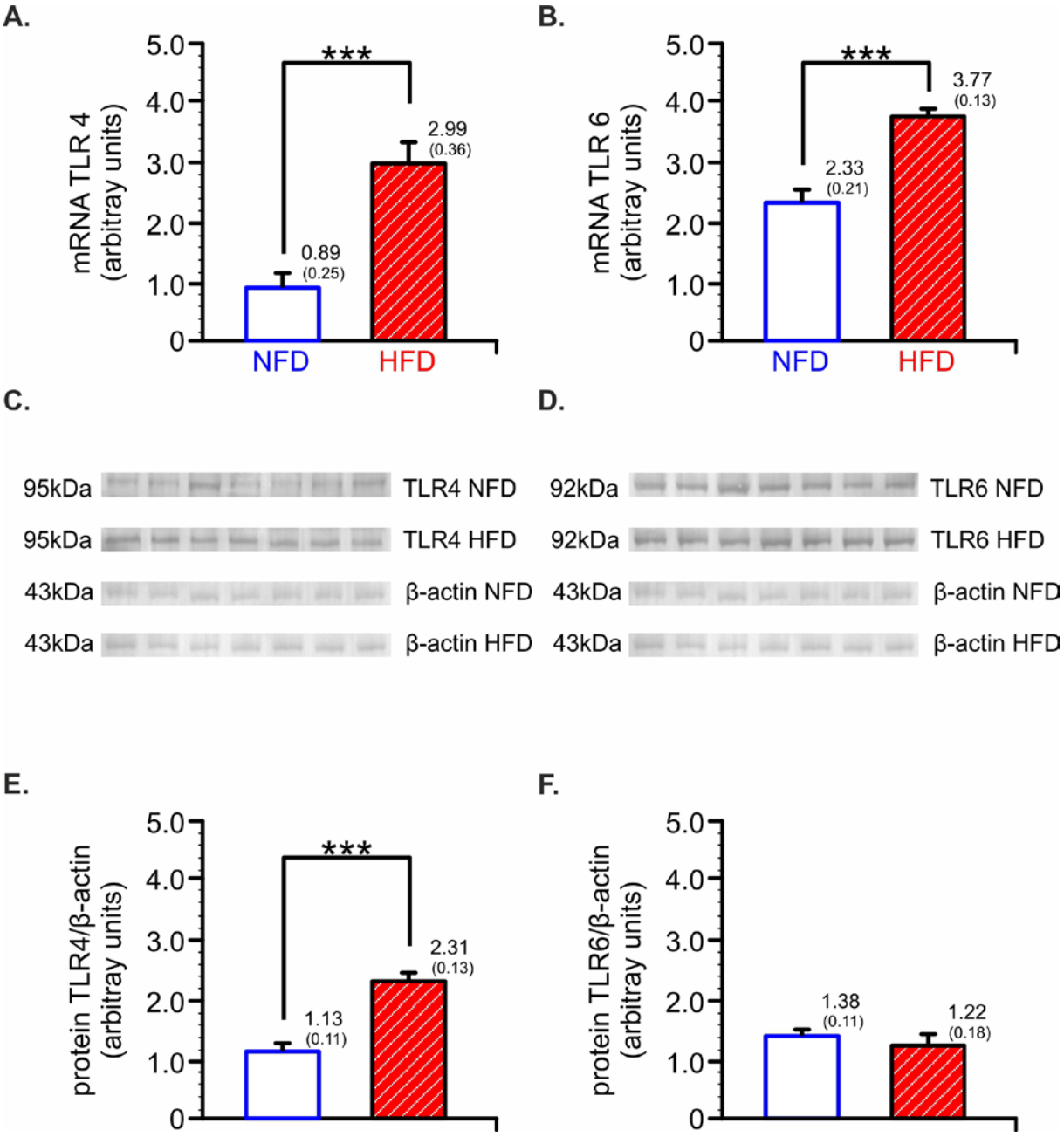

Figure 1. Expression of toll-like receptor type 4 (TLR4) mRNA (**A**) and protein (**C, E**), and toll-like receptor type 6 (TLR6) mRNA (**B**) and protein (**D, F**) in the left ventricle tissue in rats on a normal fat diet (NFD) and rats on a high fat diet (HFD). Means (SE) are shown; ****P* <0.001