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Interleukin 6 modulates PPARα and PGC-1α and is involved in high-fat diet induced cardiac lipotoxicity in mouse

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ABSTRACT

Background: Interleukin 6 (IL-6) may be involved in regulation of cardiac lipid metabolism and mitochondrial function through its influence on peroxisome proliferator-activated receptors (PPARs). In this study we evaluated the impact of the physiological level of IL-6 on the expression of PPARα and PGC-1α in the heart and the effect of lack of this cytokine on high-fat diet (HFD) induced lipotoxicity.
Methods: Male C57BL6/J wild type (WT) and IL-6 knock-out (IL-6KO) mice were used. 20 animals of each genotype were fed with HFD for 15–18 weeks. Cardiac function was assessed using echocardiography and cardiomyocyte ultrastructure was examined using electron microscopy. QT-PCR and Western blotting were applied to estimate the expression of PPARα and PGC-1α at the transcriptional and protein levels.
Results: At baseline WT and IL-6KO mice had similar size and function of the left ventricle. HFD induced similar left ventricular hypertrophic response in both groups without causing heart failure, but only WT animals had increased resting ejection fraction of the LV. Ultrastructure of HFD groups showed markers of lipotoxicity, that were more pronounced in IL-6KO group. In basal conditions IL-6KO animals had lower PPARα and similar PGC-1α expression as compared to WT. HFD induced downregulation of both PPARα and PGC-1α in WT animals, while in IL-6KO mice this effect was constrained.
Conclusion: IL-6 is involved in basal regulation of PPARα and PGC-1α expression in cardiomyocytes. The lack of this cytokine promotes high-fat diet induced lipotoxicity but without overt manifestations of cardiac failure.

1. Introduction

Metabolic syndrome, which is characterized by glucose intolerance, hypertriglyceridemia, hypertension and central obesity is associated with doubled risk of incidence of heart failure development in epidemiological studies [1]. Obesity promotes cardiovascular disease by mechanisms such as impaired glucose tolerance, diabetes, hypertension, and increased free radical formation. Chronic low-grade inflammation was identified as the underlying background of these changes and of elevated cardiovascular risk. Interleukin 6 (IL-6), a cytokine released by the fat tissue, was proposed to be one of mediators of this chronic inflammatory state [2,3]. However, recent decade has brought the evidence for the protective role of IL-6 secreted in short pulses from skeletal muscles on the development of metabolic disorders [4,5]. Physiological levels of IL-6 play an important role in the regulation of cardiac metabolism and IL-6 is required for the activation of protective mechanisms against ischemic injury of the myocardium [6]. IL-6 is involved in lipid uptake and lack of this cytokine upregulates free fatty acid, diacylglycerol and ceramide fractions in the cardiomyocytes [7]. These mechanisms are postulated to mediate development of obesity cardiomyopathy, however the exact mechanisms remain obscure [8]. Genetic modifications leading to increased myocardial fatty acid uptake result in left ventricular dilation, systolic dysfunction, and cardiac hypertrophy [8]. Also long term high feeding with chow rich in saturated fatty acids in wild type mice leads to development of cardiomyocyte hypertrophy, fibrosis and reduction of the cardiac contractility [9]. Fatty acids are the main source of energy for cardiac myocytes, but saturated fatty acids are less prone to supply the oxidative phosphorylation in these cells and cause lipotoxicity [10]. It was recently postulated that IL-6 mediates the toxicity of saturated fatty acid on rat neonatal cardiomyocytes by downregulation of peroxisome proliferator-activated receptor-α (PPAR-α) and -delta (PPAR-δ) [11]. PPARs and their cooperating particle PPAR-g coactivator1

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(PGC-1α) orchestrate the baseline long-term regulation of metabolism in cardiomyocytes, and IL-6 and other proinflammatory mediators may influence the level of PPARα. The aim of this work was to assess the impact of IL-6 on expression of the regulatory axis of PPARα and PGC-1α proteins in animals fed with diet rich in highly saturated fat.

2. Methods

2.1. Animals

Male mice devoid of IL-6 expression — C57BL/6J IL-6-/-$^{TM Kopf}$ (IL-6 KO) and from wild-type (WT) C57BL/6J (wild type) and C57Bl/J IL-6-/-$^{TM Kopf}$ mice lacking functional IL-6 gene were used in the study. Animals were obtained from the Center of Experimental Medicine of the Medical University of Bialystok and were originally purchased from Jackson Laboratories (West Grove, PA, USA). The genotype of the founder animals of the colony and randomly assigned mice from the experimental groups was confirmed using PCR as described previously [12].

Animals were kept in constant temperature of 22 °C ± 1 °C in 12:12 dark–light cycle with free access to chow and water. Baseline characteristics were performed in 4 month-old animals of both genotypes fed with the standard diet. Mice were weighed every week, daily consumption of the chow was measured every two days. A selected group of animals, not qualified for further analyses, was allowed to use the running wheel for 5 consecutive days to evaluate the spontaneous physical activity. Only sedentary mice, not subjected to voluntary wheel-running were taken for molecular and morphological analyses.

Two study groups, each containing 20 mice of either WT or IL-6KO genotype, were fed with the high-fat diet for 16 weeks starting at the age of 3–4 months. The standard diet contained 10% calories from fat, 30% calories from protein and 60% calories from carbohydrate (Labofeed H Standard, Morawski, Poland). The high-fat diet (HFD) containing 60% calories from fat, 20% from protein and 20% carbohydrate was purchased from Research Diets, Inc., NewBrunswick, NJ, USA (#D-12429). The energy content for high-fat and normal diets was 5.24 kcal/g and 3.1 kcal/g, respectively. Lard was the main source of fat in the HFD diet (317 g/kg), the cholesterol content was 279.6 mg/kg, i.e. 54.4% calories.

The experimental procedures were carried out according to the European Council Directive of 24 November 1986 (6/609/EEC) and were approved by the Local Animal Ethics Committee at the Medical University of Bialystok. The ethical issues were adherent to the statement of ethical publishing [13].

2.2. Echocardiography

Echocardiographic examination was performed in 12–15 mice from each group under the isoflurane anesthesia. Animals were placed into the anesthesia induction chamber flushed with 2% isoflurane and maintained with isoflurane at 0,7–1,5% carried by the oxygen via the face mask using the rodent Halovet vaporizer. Animals were placed on the heating pad and the coat was removed from the chest. The GE VIVD E9 echocardiographic system equipped with the i13L 14 Mhz probe was used to collect long and short axis two dimensional recordings for later evaluation. The measurements of the LV structure and function were performed offline using the EchoPAC software (GE). The measurements of the LV dimensions were performed in the anatomical M-mode and each variable was the mean from 3–5 single measurements, usually in consecutive cardiac beats. The methodology of the echocardiographic measurements and calculations is described elsewhere [14].

After echocardiography mice were sacrificed by cervical dislocation, the hearts were immediately excised, left ventricle was briefly rinsed in cold phosphate-buffered saline (PBS) and fixed in phosphate-buffered formalin for histological studies.

2.3. Histology and electron microscopy

Four animals from each group (naïve) were sacrificed, the hearts were immediately excised, flushed with ice cold PBS buffer and samples were taken for light and electron microscopy. A detailed processing of the samples for electron microscopy was described in detail in our previous work [15].

Samples for light microscopy were formalin-fixed, embedded in paraffin and cut into 5 μm sections. Slides were deparaffinized and stained with H&E for measurement of cardiomyocyte cross-sectional size.

2.4. Western blot

Samples of the left ventricle for molecular analyses (protein and mRNA) were collected from naïve animals after cervical dislocation and immediately placed in liquid nitrogen. Western blotting procedure was performed as described previously [16]. Briefly, 6 to 12 samples of protein extracts from each group were subjected to SDS-PAGE and blotted onto nitrocellulose membranes (BioRad, USA). Ponceau Red (Sigma, USA) staining was applied to control equal sample loading, as it was validated as an alternative to actin blotting [17]. The following primary antibodies were purchased from SantaCruz Biotechnology: anti-PPARα (sc-9000), anti-PGC-1 (sc-13067), anti-citrate synthase (sc-242444); anti-cytochrome c was purchased from Cell Signaling (#4272) and anti-cytochrome c oxidase (IV) from Abcam (ab16056). Secondary antibodies conjugated with horseradish peroxidase were anti-rabbit Star54 (Serotec, Oxford, UK) or anti-goat (SantaCruz Biotechnology, #sc-2033). Blots were visualized using enhanced chemiluminescence reaction (ECL Pico, Pierce, Rockford, IL, USA), and exposed on the x-ray film (X-Omat Blue, Kodak, Rochester, NY). To remove antibodies bound to the membrane before re-probing for a next protein, the blots were incubated with the Restore®Western Blot Stripping Buffer (Thermo Scientific). Films were scanned and quantified using the Image J software (National Institutes of Health, USA). The results of particular experiments were related to the expression of proteins in the WT control group, which was set as 1.

2.5. qRT-PCR

LV samples (ca. 30 mg in weight) were crushed in liquid nitrogen and RNA was extracted using commercially available kits Nucleospin RNA II from Macherey-Nagel (Germany), according to the supplied protocol for fibrous tissues. Reverse transcription was performed using High-Capacity cDNA Archive Kit from Applied Biosystem (USA). The quantitative real-time PCR (qRT-PCR) was carried out in TaqMan 7900 system (Applied Biosystem, USA) using primers, probes and reagents supplied by the manufacturer (PPARα: Mm00440939_m1, PGC1: Mm01208835_m1 and GPDH: Mm99999915_g1). The expression of each analyzed gene (the number of cycles necessary to obtain the cut-off point — Ct value) was related to the expression of the GPDH. Then the $2^{-\Delta\Delta Ct}$ method with GPDH gene as internal control and young WT animal as internal calibrator was used to present changes in gene expression [18].

2.6. Statistical analysis

Statistical analysis was performed using Statistica 10 (StatSoft, USA). A post-hoc analysis of variance with Bonferroni correction and non-parametric t-tests was used in analyses and $p < 0.05$ was considered significant. The numerical data is presented as mean value ± standard deviation.