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Interleukin-6 deficiency facilitates myocardial dysfunction during high fat diet-induced obesity by promoting lipotoxicity and inflammation

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ABSTRACT

Objective: Obesity is associated with metabolic disorder and chronic inflammation that plays a crucial role in cardiovascular diseases. IL-6 is involved in regulating obesity-related lipid metabolism and inflammation. In this study, we sought to determine the role of IL-6 in high-fat diet (HFD)-induced cardiomyopathy and explore the signaling pathway.

Methods: Female, 5-week-old IL-6 knockout (KO) and littermate mice were fed a normal diet (ND, 10% fat) or HFD (45% fat) for 14 weeks. At the end of treatment, cardiac function was assessed by echocardiography. Adipose tissues and plasma were collected for further measurement. Immunohistology of CD68 was performed to detect inflammation in the heart. Masson's trichrome staining and Oil Red O staining was applied to evaluated cardiac fibrosis and lipid accumulation. Real-time PCR and Western immunoblotting analyses on heart tissue were used to explore the underlying mechanism.

Results: IL-6 KO mice displayed increased insulin resistance compared to WT mice at baseline. When fed HFD, IL-6 KO mice showed decreased gains in body weight and fat mass, increased insulin resistance relative to IL-6 KO mice feed ND. Furthermore, IL-6 KO mice developed cardiac dysfunction during HFD-induced obesity. Histological analysis suggested increased lipid accumulation, fibrosis and inflammation without affecting cardiac morphology during HFD treatment in the heart of IL-6 KO mice. Finally, IL-6 deficiency increased the phosphorylation of AMPK and ACC in the heart during HFD-induced obesity.

Conclusion: Our results suggest that IL-6 contributes to limit lipid metabolic disorder, cardiac hypertrophy, fibrosis, inflammation and myocardium lipotoxicity during HFD-induced obesity.

1. Introduction

Clinical and experimental evidence has demonstrated that obesity and obesity-related disorders, such as type 2 diabetes, hypertension and dyslipidemia, are the leading and growing factors contributing to myocardial remodeling and dysfunction that lead to high cardiovascular mortality in the world [1–3].Obesity is usually characterized by increased exacerbated inflammation accompanied by infiltration of immune cells into adipocytes [4–6]. Inflammation contributes to the development of insulin resistance, atherosclerosis, cardiomyopathy, hypertension [7,8] and their associated features during obesity [9–13]. Several genes mutated in mouse models, such as leptin [14], melanocortin-4 receptor genes [15], CARD9 [16] as well as IL-6 [17,18] are proved to be highly related to body fat regulation and myocardial function during obesity.

Interleukin-6 (IL-6) is a biologically active substance that is

Abbreviations: IL-6, interleukin-6; ND, normal diet; HFD, high fat diet; FAs, fatty acids; CD36, cluster of differentiation 36; MCP-1, monocyte chemoattractant protein 1; PPARα, peroxisome proliferator-activated receptor α; CPT-1, carnitine palmitoyltransferase 1; EndoG, endonucleus G; mTFA, mitochondrial transcription factor A; NRF-1, nuclear respiratory factor 1; EF, ejection fraction; FS, shortening fraction; LV vol-d, left ventricular volume at diastolic; LVID-d, left ventricular internal diameter at diastolic; LVPW, left ventricular posterior wall; IVS, interventricular septum; LV Mass, left ventricular mass

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expressed in various mammalian cells and tissues including adipocytes, cardiac myocytes and skeletal muscles [19,20]. IL-6 deficiency is suggested to be an important component of obesity-related insulin resistance by a number of studies [17,21]. Researches demonstrated that mice with IL-6 deficiency developed mature-onset obesity and obesity-related metabolic disorders which could partly be reversed by IL-6 replacement [20], suggesting a strong link between IL-6 and lipid metabolism. In addition, growing evidences demonstrated that serum levels of inflammation markers, including TNFα, IL-1β and IL-6 are elevated in patients with type 2 diabetes [22–24]. However, IL-6 also has important anti-inflammatory properties due to its paradox role, such as down-regulation of the pro-inflammatory cytokines tumor necrosis factor α (TNFα), and interferon γ (INFγ) during acute-phase reaction [25]. Several studies proved that high fat diet-induced obesity causes insulin resistance, inflammation, apoptosis, disorder of metabolism as well as cardiomyopathy, and these phenomena were worsened in IL-6 deficient mice [21,26].

During development, hearts shift away from the utilization of glucose and rely almost completely on fatty acids (FAs) as the energy source [27]. Therefore, the heart relies heavily on the circulating FAs due to its limited capacity for de novo synthesis of FAs [28]. Translocation of FAs from blood to cardiomyocytes depends on FA transporters including cluster of differentiation 36 (CD36) [29]. About 75% of the translocated FAs are transferred to mitochondria for the generation of ATP and the rest are converted to intracellular TAG for future use [30]. In the cytosol, FAs are activated to fatty acyl Co-enzyme A (CoA), then carnitine palmitoyltransferase 1 (CPT-1) plays essential roles in transporting fatty acyl-CoA across mitochondrial membrane into the matrix for β-oxidation. PPARα augments transcription of CPT-1 [31,32] and increases conversion of fatty acyl-CoA in the mitochondrial matrix to acetyl CoA, thus plays critical roles in metabolic regulation in diabetic hearts [27].

It is accepted that metabolic disturbance induce subcellular low-grade inflammation in the heart during the development of obesity [33,34]. Nevertheless, the precise mechanism of cardiac function and lipid metabolism, and its correlation with IL-6 in high fat diet-induced diabetes remains elusive. In the present study, we hypothesized that IL-6 deficiency would lead to myocardial lipid metabolism alteration during HFD-induced obesity, worsening cardiac lipotoxicity and dysfunction caused by HFD. To test this hypothesis, by using IL-6 knockout (IL-6 KO) mouse model fed with fat-enriched diet, we first assessed integral lipid metabolism. We then studied cardiac function, myocardial fibrosis, inflammatory infiltration and lipid accumulation. Finally we examined the cardiac expression of genes related to fatty acid metabolism. From the results we proposed the possible mechanisms underlying IL-6 and cardiomyopathy associated with lipid accumulation.

2. Materials and methods

2.1. Animals

The investigation with experimental animals conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was evaluated and approved by the Experimental Animal Ethic Committee of China Pharmaceutical University. The age-matched female IL-6 knockout (KO) mice and wild type (WT) littermates were purchased from Model Animal Resource Information Platform of Nanjing University (#D000054) at the age of 4-week-old and were used in the study. Mice were housed in constant temperature of 22 ˚Cin a 12/12-light/dark cycle with free access to regular rodent chow and tap water. The genotype of the founder animals of the colony and randomly assigned mice from the experimental groups was confirmed using PCR according to the protocols.

Mice had access to regular chow and tap water and were allowed to adjust to the environment for at least 1 week. Then the regular chow (Nanjing University, China. Protein 20 kcal%; carbohydrate 70 kcal%; fat 10 kcal%) was replaced by a high-fat diet containing 45% fat (Research Diets, USA, D12451. Protein 20 kcal%; carbonhydrate 35 kcal%; fat 45 kcal%).

The treatment groups were as follows: WT + vehicle; IL-6 KO + vehicle; WT + high fat diet; and IL-6 KO + high fat diet. Treatment was continued for 14 weeks.

2.2. Echocardiography

Mice were anesthetized with isoflurane and a small animal ultrasound systemVevo2100 (VisualSonics, Toronto, Canadaia) equipped with a 30MHzpediatric transducer was used to record a two-dimensional and M-mode echocardiogram. The transducer was placed on the chest (using transducer gel as an offset) and a transthoracic echocardiogram was externally recorded. The echocardiographer was blinded to the genotype of the animal. From the M-mode echocardiogram, software on the Vevo 2100 machine was used to measure the thickness of the left ventricular posterior wall (LVPW) and the internal diameter of the left ventricle in end-diastole (LVID-d) and end-systole (LVID-s). These values were used to automatically calculate the mass of the left ventricular (LV Mass), the percentage of ejection fractions (EF) and fractional shortening (FS), and the volume of the left ventricle in end-diastole (LV Vol-d) and end-systole (LV Vol-s) by Vevo 2100 software.

2.3. Metabolic parameters

At the end of the experiment, mice were euthanized with an intraperitoneal injection of chloral hydrate. Blood samples were collected and plasma TG and TC levels were determined using GPO-PAP method (Biosino Biotechnology and science Inc., Beijing, China). HDL-C and LDL-C were measured using direct method (Biosino Biotechnology and science Inc., Beijing, China).Heart tissue was homogenized and LDH activity was determined using LDH assay kit (Jiancheng Biotechnology, Nanjing, China).

2.4. Immunohistochemistry

4% paraformaldehyde-fixed, paraffin-embedded tissue blocks were sectioned into 5 μm slices. After deparaffinization and rehydration, the antigen retrieval was performed using microwave antigen repairing method and the endogenous peroxidase activity was blocked with 3% H_2O_2. And then sections were blocked with 10% rabbit serum or 3% bovine serum albumin (BSA) in phosphate buffered saline (PBS) for 30 min at room temperature. The primary antibodies against CD68 (dilution 1:200; Abcam, ab955) were applied overnight at 4 ˚C. After 30 min of washing, the sections were incubated with proper secondary antibody (Servicebio, GB23303) conjugated with horseradish peroxidase (HRP) at dilution 1:200 for 1 h at room temperature. In the next step sections were rinsed with PBS and incubated with 3, 3′-diaminobenzidinetetrahydrochloride (DAB, Dako, Copenhagen, Denmark) for 10 min. Nuclei were counterstained with hematoxylin. Negative control of staining was performed in the corresponding sections by omitting the primary antibody. All of slides were scanned by the digital slide scanner NanoZoomer 2.0RS (Hamamatsu, Massy, France), which allowed an overall view of the samples.

2.5. Histological Assessment

Sections measuring 5 μm were cut from paraffin-embedded tissue blocks and double-stained with hematoxylin (to stain the nuclei, blue) and eosin (to outline the cardiomyocyte sectional area, red) as the H & E staining. Masson staining for the presence of interstitial collagen fiber accumulation was a marker of cardiac fibrosis and was performed by using Masson Staining Kit (Servicebio, Wuhan, China). These sections