Nutrition, Metabolism & Cardiovascular Diseases (2013) 23, 566–573

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Nutrition, Metabolism & Cardiovascular Diseases

Effects of exercise training on cardiac apoptosis in obese rats☆

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Received 21 April 2011; received in revised form 10 October 2011; accepted 2 November 2011
Available online 7 March 2012

KEYWORDS
Physical activity;
Heart;
Caspase;
Cell death;
Obese

Abstract *Background and Aim:* The purpose of this study was to evaluate the effects of exercise training on cardiac apoptotic pathways in obesity.
Methods and Results: Sixteen lean Zucker rats (LZR) and sixteen obese Zucker rats (OZR) of 5–6 months of age as well as the other sixteen obese rats were subjected to treadmill running exercise for 1 h everyday for 3 months (OZR-EX). After exercise training or sedentary status of the rats, the excised hearts from the three groups were measured by heart weight index, H&E staining, TUNEL assays and Western blotting. Cardiac TUNEL-positive apoptotic cells, the protein levels of TNF alpha, Fas ligand, Fas receptors, Fas-associated death domain (FADD), Bad,

☆ Supported by the National Science Council, Taiwan (NSC 95-2314-B-039-040).
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0939-4753/$ - see front matter
doi:10.1016/j.numecd.2011.11.002

Bax, activated caspase 8, activated caspase 9, and activated caspase 3 were higher in OZR than those in LZR. The protein levels of TNF alpha, Fas ligand, Fas receptors, FADD, activated caspase 8, and activated caspase 3 (Fas pathway) and the protein levels of Bad, Bax, Bax-to-Bcl2 ratio, activated caspase 9, and activated caspase 3 (mitochondria pathway) were lower in OZR-EX than those in OZR.

Conclusion: Cardiac Fas-dependent and mitochondria-dependent apoptotic pathways become more activated in obesity. Exercise training can prevent obesity-activated cardiac Fas-dependent and mitochondria-dependent apoptotic pathways. Our findings demonstrate a new therapeutic effect of exercise training to prevent delirious cardiac Fas-mediated and mitochondria-mediated apoptosis in obesity.

Introduction

The obese Zucker rat presents various similar cardiopulmonary deficits observed reported in obese humans, including respiratory dysregulation, chest wall limitation, upper airway narrowing, hypertension, myocardial hypertrophy, and poor exercise capacity [1—4]. Severe obesity has been recognized as one of the causes of cardiomyopathic disorders manifesting chronic volume overload, left ventricular hypertrophy and the development of heart failure [5—8].

The occurrence of apoptosis has been reported to be responsible for the loss of cardiomyocytes and is recognized as a predictor of adverse outcomes in cardiac diseases or heart failure [9—11]. The 'extrinsic' Fas receptor-dependent (type I) apoptotic pathway is initiated by binding the Fas ligand to the Fas receptor, leading to the formation of a death-inducing signal complex initiating recruitment of the Fas-associated death domain (FADD) of the adaptor protein [12]. The activated caspase 8 cleaves pro-caspase 3, which further undergoes autocatalysis to form active caspase 3, a principle effector caspase of apoptosis [13,14]. Additionally, activated caspase 8 can cleave Bcl2 homology domain 3 (BH3)-interfering domain death agonist (Bid), and the cleaved Bid to t-Bid then causes release of the mitochondrial cytochrome *c*, further activating pro-caspase 9, that activates pro-caspase 3 [12,15]. The t-Bid is one of the significant key components involved in the intracellular molecule signaling from Fas-dependent apoptotic pathway to the mitochondria-dependent apoptotic pathway [12,15].

The 'intrinsic' mitochondria-dependent (type II) apoptotic pathway is mediated by internal factors, especially in the mitochondria [12]. Bcl2, an anti-apoptotic protein, prevents cytochrome *c* release whereas Bax, pro-apoptotic proteins, enhance cytochrome *c* release from the mitochondria [12]. When the cytochrome *c* is released from the mitochondria into cytosol, it is responsible for caspase 9 activation, which further activates the caspase 3 and executes the apoptotic program [16]. The increased cardiac apoptosis was found in leptin-deficiency and leptin-resistant mice [17]. In addition, our previous studies indicate that Fas receptor-dependent and mitochondria-dependent apoptotic pathways are more activated in obese Zucker rats than those in lean rats [18,19].

Exercise can induce cardioprotection against myocardial ischemia-reperfusion injury [20] and can prove to be an important rehabilitation program for patients with heart failure [21]. A previous study has reported that the exercise training reduces Bax protein levels, caspase activity, and DNA fragmentation in cardiac muscles without any change in apoptosis of skeletal muscle in the obese rats [22]. The effects of exercise training on Fas ligand, Fas receptor, FADD, caspase 8, and TUNEL-positive apoptosis in obese hearts is still unidentified. In the current study, we hypothesized that exercise training may prevent activated Fas-mediated and mitochondria-mediated cardiac apoptosis in obesity.

Methods

Animal model

The study is performed using 16 lean (Fa/Fa or Fa/fa) and 32 obese (fa/fa) age-matched from 2 to 3 months old male Zucker rats. The animals were bred by the second generation of Zucker breeders purchased from Charles River Lab, France. Ambient temperature was maintained at 25 °C and the animals were kept on an artificial 12 h light—dark cycle. The light period started at 7:00 A.M. Rats were provided with standard laboratory chow (Lab Diet 5001; PMI Nutrition International Inc., Brentwood, MO, USA) and water *ad libitum*. The protocols were approved by the Institutional Animal Care and Use Committee of China Medical University and were followed "Principles of laboratory animal care" (NIH publication No. 86—23, revised 1985).

Exercise training

Sixteen lean rats were kept in sedentary condition and thirty-two obese rats were randomly divided into sedentary and exercise training groups, i.e. the sedentary lean group (LZR), the sedentary obese group (OZR), and the obese group after exercise training (OZR-EX). Rats in the exercise group were trained on a motor-driven treadmill at the speed of 12 m/min for 20 min on the first day of the experiment. One week later, running time gradually extended to 60 min/day. The running speed was then increased 3 m/min every week progressively until 27 m/min through the training protocol. These rats were trained 5 days/week for 12 weeks continuously. Animals in the sedentary control group were placed on the treadmill without exercise training regimen for 20 min everyday. To avoid acute effects of exercise, they were sacrificed 48 hr after exercise training.

Biochemical analysis

Triglycerides, IL-6, and leptin were measured by spectrophotometry and ELISA. The citrate synthase activity was assayed by homogenizing weighed soleus muscle samples in HES buffer and was determined spectro-photometrically [23].