High-intensity Interval versus Mild-intensity Endurance Training in Rats Fed a High-fat or Control Diet

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Abstract

The current study was to compare the effects of high-intensity interval training (HI) to mild-intensity endurance training (ME), combined with a high-fat diet (HFD) or control diet (CD), on metabolic phenotype and serum corticosterone levels in rats.Although exercise is linked to benefits of metabolic health, it is surprising that few studies have explored the stress responses and interaction of different exercise regimens and HFD feeding, either independently or interactively.It remains unclear whether different exercise regimens lead to similar metabolic and stress responses and adaptations in the presence of an HFD.

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Protocol

Animals and housing conditions breeding

Step 1.

Fifty-three male Sprague-Dawley rats (8–10 weeks of age, weighing 190 ± 15 g) were supplied by a local breeding facility (Wuhan University Center for Animal Experiment/A3-Lab, Wuhan, Hubei Province, China). Rats were housed three to four per cage under standard conditions: constant temperature (23 ± 1°C) and humidity (40–60%), *ad libitum* access to food and water, and 12 h light–dark cycle (lights on 19:00–07:00). Upon arrival, rats were acclimated to environment and circadian rhythm for 1 week prior to intervention. Rats were randomly assigned into six groups according to diet and exercise protocol, as follows. Control diet and sedentary (CS, n = 11), control diet with mild-intensity endurance training (CME, n = 8), control diet with high-intensity interval training (CHI, n = 8), high-fat diet and sedentary (HS, n = 10), high-fat diet with mild-intensity endurance training (HME, n = 8), and high-fat-diet with high-intensity interval training (HHI, n = 8). Rats were pair-fed with either an HFD (D12451; 4.73 kcal/g, energy content: 45% fat, including 12.3% from soya oil and 87.75% saturated fat from lard; 35% carbohydrates, including 21.1% from corn starch, 28.9% from Maltodextrin 10, and 50% from sucrose; and 20% protein) or CD (D12450B; 3.85 kcal/g, energy content: 10% fat, including 55.6% from soya oil and 44.4% saturated fat from lard; 70% carbohydrates, including 45% from corn starch, 5.0% from Maltodextrin 10, and 50% from sucrose; and 20% protein) (Research Diets, Inc. New Brunswick, NJ, United States). These matched and purified ingredient-based diets are the standard for research in the field of obesity, T2DM, and metabolic syndrome. Rats were weighed twice per week and their food was changed twice daily. Daily food intake was measured by weighing total amount of food provided to the rats and subtracting the remaining food in the cage every 24 hours. Cage bedding was searched manually for leftover pieces

of pellets to take into account any food spillage and hoarding. All experimental procedures and facilities were operated in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Treadmill training protocols

Step 2.

Training was performed on a specialized treadmill that had a motor-driven grade and time setting (Zhenghua Biological Equipment Company, Anhui, China). The treadmill consisted of eight parallel runways (each running track = 100 cm × 9.6 cm × 12 cm) with two transparent outer covers (50 cm × 88 cm), which prevented the rats from falling off the treadmill. Animals in the exercise groups were acclimated to the treadmill for 15 min/d at a speed of 5 m/min for 1 week before starting the exercise program. In accordance with nocturnal habits, all rats were trained during the dark cycle (07:00–11:00 AM). Rats attempting to rest were encouraged to continue running by gently tapping the feet with a bristle brush on the rear grid. After acclimation and 2 days of rest, rats in the exercise groups underwent training 5 days/week for 10 weeks with matched running distances. Exercise intensity was performed and adjusted, as described previously (Bedford TG, et. 1979). Animals were placed on their respective dietary and training regimens for 10 weeks. The running speed for ME training started at 10 m/min (5-degree inclination), was increased by 2 m/min per week over the first 4 weeks, and then maintained at 16 m/min for the remaining 6 weeks. Duration of the constant, mild intensity of ME training was of 40 min/day. The HI groups trained in interval sessions consisting of successive 30 s periods of heavy intensity interspersed with 10 s of sedentary recovery. Each session began at a speed of 20 m/min (5-degree inclination), was increased by 4 m/min per week over the first 4 weeks, and maintained at 32 m/min for the remaining 6 weeks. Duration of HI training was of 20 min/day. To account for the stress induced by animal handling, sedentary groups also were placed in a stationary treadmill for acclimation.

Blood sampling and serum analyses

Step 3.

After 10 weeks, animals were fasted overnight, and then anesthetized with pentobarbital sodium (40 mg/kg, i.p.). After the animals were completely anesthetized, the abdominal fur was shaved off and the abdominal cavity was rapidly opened along the medial line. Blood was rapidly drawn from the abdominal aorta using vacuum tube. All samples were collected between 08:00 am and 10:00 am to avoid temporal influences on blood metabolites. Serum was collected after samples were centrifuged at 3000 rpm at 4°C for 15 minutes. Serum samples were collected and fasting glucose, triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured using an automated analyzer (AEROSET Automatic Biochemical Analyzer, Abbott Company, IL, USA). Commercially rat-specific enzyme-linked immunosorbent assay (ELISA) kits were used to determine serum insulin (Cat. No. EZRMI-13K, Linco Research, St. Charles, MO, USA) and corticosterone level (Cat. No. E-2724, Shanghai Meilian Bio-tech Company, China). The quantitative insulin sensitivity check index (QUICKI) was calculated according to the formula, as described previously QUICKI = 1/ [log (*I0*) + log (*G0*)], where *I0* is fasting insulin (IU/ml) and *G0* is fasting glucose (mg/dl) (Muniyappa R, et. 2009).

Tissue collection and histological analysis

Step 4.

Livers and fat pads in the mesenteric (MES), retroperitoneal (RET), and epididymal (EPI) regions were

collected and weighed. Liver samples were fixed in 10% neutral buffered formaldehyde, embedded in paraffin, and sectioned at a thickness of 4 µm on a microtome (Leica RM 2016, Leica Biosystems, Wetzlar, GER) for standard hematoxylin-eosin (H&E) staining. Histological samples were imaged using an electron microscope (Olympus DP72, Olympus Corporation, Tokyo, Japan).