SUPPLEMENTAL METHODS

Cardiorespiratory fitness

Cardiorespiratory fitness (CRF) was measured using a treadmill exercise test modified from a previous study [1]. CRF level was reported as the best running distance among 5 consecutive days of the test [2].

Whole-body substrate oxidation during vigorous exercise

Whole-body substrate, including carbohydrate and fatty acid, oxidation during vigorous exercise was evaluated using indirect calorimetry during a treadmill exercise test as detailed in a previous study [3].

Plasma insulin sensitivity and lipid profiles

Fasting plasma glucose was determined using a colorimetric assay kit. Insulin level was measured using a sandwich enzyme-linked immunosorbent assay (ELISA). Homeostatic model assessment for insulin resistance was calculated using the equations previously detailed elsewhere [4]. Triglyceride and total cholesterol levels were evaluated using colorimetric assay kits. Plasma high density lipoprotein level was analyzed using a commercial assay kit. Low density lipoprotein level was estimated using Friedewald's equation [5].

Echocardiography and heart rate variability

Echocardiography was performed to evaluate systolic and diastolic cardiac function. Heart rate variability (HRV) was done to determine the autonomic cardiac function. Each step of echocardiography and HRV were detailed in a prior study [6].

Open-field test

The open-field test was performed to evaluate anxiety and general locomotive activity. The apparatus used for this assessment consisted of a circular-based box with the opening at the top (70 cm diameter and 50 cm high). The rat was placed into the middle of the box and allowed 10 minutes to explore. The distance and speed in the area of the open-field was counted through the camera and analyzed with the Smart version 3 program (PanLab Harvard Apparatus, Holliston, MA, USA).

Modified novel object location and recognition tests

Novel object location and recognition tests were assessed in order to determine hippocampal dependent and independent cognitive functions. The protocol was described in a previous study [7].

Malondialdehyde concentration in cardiac and brain tissues

Malondialdehyde concentration in the left ventricular cardiac tissues and the whole brain were measured using high-performance liquid chromatography (Thermo scientific, Bangkok, Thailand). Each step of the analysis was detailed in a prior study [3].

Protein expression analyses

Protein expressions in left ventricular and hippocampal tissues were measured by Western blot, as previously described elsewhere [3]. The full Western blot for cardiac and brain protein expressions were shown in the Supplemental Figs. S4, S6 respectively.

Cardiac mitochondrial function and mitochondrial respiration assay

The cardiac mitochondria from the left ventricle were isolated by the differential centrifugation [8] to enable the determination of mitochondrial reactive oxygen species (ROS) level, mitochondrial membrane potential change, mitochondrial swelling, and mitochondrial respiration. The details of all assays were described in a prior study [3].

Hippocampal ROS level

2′-7′dichlorofluorescin diacetate (DCFH-DA) dye was used to determine hippocampal ROS production, as detailed in a previous study [9].

Ex vivo brain incubation with regular insulin solution

The whole brain was incubated with human regular insulin solution to determine the expressions of insulin signaling-related proteins by Western blot. Those proteins were phosphorylated-insulin receptor substrate 1 (p-IRS1), total IRS1, p-Akt, and total Akt. The details were shown in a previous study [10].

Apoptotic cell death assay

Apoptotic cell death of left ventricular and hippocampal tissues were determined using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, as described in a prior study [11].

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