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Cardiac, skeletal muscle and serum irisin responses to with or without water exercise in young and old male rats: Cardiac muscle produces more irisin than skeletal muscle

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ABSTRACT

Irisin converts white adipose tissue (WAT) into brown adipose tissue (BAT), as regulated by energy expenditure. The relationship between irisin concentrations after exercise in rats compared humans after exercise remains controversial. We therefore: (1) measured irisin expression in cardiac and skeletal muscle, liver, kidney, peripheral nerve sheath and skin tissues, as also serum irisin level in 10 week-old rats without exercise, and (2) measured tissue supernatant irisin levels in cardiac and skeletal muscle, and in response to exercise in young and old rats to establishing which tissues produced most irisin. Young (12 months) and old rats (24 months) with or without 10 min exercise (water floating) and healthy 10 week-old Sprague-Dawley rats without exercise were used. Irisin was absent from sections of skeletal muscle of unexercised rats, the only part being stained being the perimysium. In contrast, cardiac muscle tissue, peripheral myelin sheath, liver, kidneys, and skin dermis and hypodermis were strongly immunoreactivity. No irisin was seen in skeletal muscle of unexercised young and old rats, but a slight amount was detected after exercise. Strong immunoreactivity occurred in cardiac muscle of young and old rats with or without exercise, notably in pericardial connective tissue. Serum irisin increased after exercise, being higher in younger than older rats. Irisin in tissue supernatants (cardiac and skeletal muscle) was high with or without exercise. High supernatant irisin could come from connective tissues around skeletal muscle, especially nerve sheaths located within it. Skeletal muscle is probably not a main irisin source.

1. Introduction

Skeletal muscle produces an exercise-induced hormone, irisin, which is released into the circulation, especially during or immediately after physical activity. It converts white fat into brown fat, enhancing metabolic uncoupling and hence caloric expenditure [5]. Brown adipose tissue dissipates energy stored in triglycerides as heat via an uncoupling protein, UCP1 [4]. Thus irisin could be a new uncoupling agent (like dinitrophenol) that can deplete the body of ATP and increase heat production [5].

Irisin is a product of a fibronectin type III domain-containing 5 (FNDC5). FNDC5 expression is induced by exercise training, and exogenous FNDC5 induces uncoupling protein 1 (UCP1) expression in subcutaneous white adipocytes [5]. Timmons et al. [23] reported that FNDC5 is induced in muscle in only a minority of subjects, whereas all types of training programs led to enhanced cardiovascular function in the vast majority of people; indeed, plasma irisin was elevated only in highly active elderly subjects [23], and doubles Fndc5 mRNA expression in the muscle of older and obese subjects after a 10-week protocol of endurance exercise [5]. Exercise training in pigs does not increase FNDC5 mRNA or protein in the deltoid or triceps brachii of FHM or normal pigs, while increasing

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circulating irisin only in the FHM pigs [10]. Hecksteden et al. [12] also reported that training does not cause an increase in circulating irisin concentration in subjects exercised 3 times per week for 26 weeks. Furthermore, Raschke et al. [20] showed that FNDC5 mRNA expression in muscle biopsies from 2 different human exercise studies was unchanged by endurance or strength training, concluding that it is unlikely that the beneficial effect of irisin seen in mice will also occur in humans.

If it does, some important questions arise. First, is irisin produced in skeletal muscle, or is cardiac muscle its major source? Second, if irisin is produced in skeletal muscle, why is there not a marked increase in young subjects after endurance exercise as seen in older subjects? Third, on the basis of the reported data, is irisin induction after endurance exercise age-dependent? Fourth, are muscle tissues resistant to injury and release of irisin peptide in young subjects, so that irisin is not released into the circulation after exercise? Fifth, is the question of whether irisin is regulated by exercise to shed more light on the functional aspects of this hormone? And whether the increase in plasma irisin has a different source? The last and most important questions are: what are the major irisin-producing tissues, skeletal muscle, cardiac muscle or others (e.g. liver, kidneys)? And is production also induced by endurance exercise?

As stated above, the exact relationship between irisin concentration after exercise in animals when compared with irisin concentration humans is still under discussion and controversial [5,10,12,18,20,23]. Serum irisin concentration increased with also ages increases with endurance exercise [23]. In this research, we wished to see how irisin concentration changed in young and old rats after exercise. This was hopefully to compare with previously reported irisin increase after endurance exercise in humans. Rats rapidly become sexually mature at ~6 weeks of age [1]. In adulthood, every month is approximately equivalent to 2.5 human years [1].

Based on the above information, we examined first the distribution of irisin in striated muscle tissues (cardiac and skeletal muscles), liver, kidneys, and peripheral nerve sheath tissues by immunohistochemistry, and serum irisin by an enzyme-linked immunosorbent assay (ELISA) in 10 week-old rats (2.5 months old rats) without exercise, and also immunohistochemically screened the distribution of irisin in the striated muscle tissues (cardiac and skeletal muscles) of the young (12 months old rats) and old healthy male rats (24 months old rats) in response to 10 min water floating (exercise). Liver, kidney, cardiac and skeletal muscle tissue supernatant and serum irisin concentration were measured by ELISA in rats with or without 10 min water floating. The goal was to see which of these striated muscle tissues (cardiac and skeletal muscles) are the best irisin producer in 2.5 months old rats without exercise, compared with 12 month and 24 month rats responses with or without 10 min water floating exercise.

2. Materials and methods

Experiments involving the animals were conducted according to the policy of the European convention for the protection of vertebrate animals used for experimental and other scientific purposes in accordance with "Recommendations on the Establishment of Animal Experimental Guidelines," and ethical procedures were conducted under Reduction, Replacement and Refinement (the 3 Rs rule). The study was carried out at the Experimental Research Unit of Firat University (FUDAM). Rats rapidly develop and become sexually mature at ~6 weeks of age. In adulthood, every month of the animal is equivalent to ~2.5 human years. The average laboratory rat lives ~3 years [1].

Sprague-Dawley type male rats (260–380 g) were housed under a controlled temperature of 21 °C (±10%) and a humidity of 65% (±5%) with a 12 h day/night cycle. They were fed standard laboratory chow (commercial standard pellets) ad libitum. Each group composed of six Sprague-Dawley type male rats. Rat at 10 weeks of age were randomly assigned, young rats at 12 months of age (n:6); young rats (n:6) received 10 min water floating (stressful exercise); old rats at 24 months (n:6) received the same treatment. After overnight fasting, rats (except for control rats) were exercised in a cylindrical polystyrene container with water at 24–26 °C as previously reported [24,25]. The control rats were also kept at 24–26 °C for 10 min in the cages.

After exercise, rats were decapitated under ketamine-HCl (75 mg/kg) and 10 mg/kg xylazine-HCl anesthesia. To reduce the effect of circadian rhythm, experiments began at 08.00 AM, and were completed by 11.00 AM. The heart, skin, liver, kidneys and gastrocnemius tissue from each rats were excised, cleaned, divided into 2 pieces [one for immunohistochemistry (IHC)] and the other for homogenization for supernatant), washed in ice-cold saline, and tissue samples for IHC staining were immediately placed in 10% formaldehyde. For tissue homogenate, 100 mg were taken from each tissues and placed a tube that with 500 KIU aprotinin before being carefully homogenized in Phosphate-Buffered Saline (PBS, pH 7.4) solution according to published methods [3]. Tissue homogenizations have done twice. Blood was collected in test tubes, and processed for serum preparation by 5 min centrifugation at 4000 rpm; tissue homogenates were centrifuged at 4000 rpm for 10 min. Tissue and serum were measured using a commercially available Rat irisin ELISA Kit (EK-067-52, Phoenix, USA). Intra-assay and inter-assay were <4–6%, and <8–10%, respectively. The quantitation range was between 0.066 and 1024 ng/mL assay. Irisin concentration in the supernatant was assayed as previously published [2,3].

Irisin expression in the tissues was detected immunohistochemically with Avidin-Biotin-peroxidase Complex (ABC) as per Hsu et al. [13] with minor modification [16], except for the primary antibody step, which was a primary antibody is from Phoenix Pharmaceuticals, Inc., CA, USA (cat no: H-067-17). This was highly specific against the protein being examination and did not cross-react with related proteins. Slides were with hematoxylin and covered with lamella to make them into permanent preparations. They were examined under a light microscope (Olympus BX 50 Olympus Corporation, Tokyo, Japan) and photographed.

2.1. Statistical analysis

Statistical analysis used SPSS (Version 10; SPSS, Chicago, IL). The Kolmogorov–Smirnov test was used for normality of distribution, and the variables were normally distributed. Correlation within groups was calculated using Pearson correlation analysis. Tissue supernatant and serum data are expressed as means ± SD. $p < 0.05$ was considered significant.

3. Results

Close to the ending of 10 min exercise only the head of the rats were above water. Old rats floated less well than young rats floating by the end of each experiment. The minimal irisin detection level in the supernatant of tissues was 0.072 ng/mL (cf. 0.066 ng/mL according to Phoenix's own assay). Dilution of supernatant samples has not been also affected. Intra assay and inter assay were lower than <7 and <10%, respectively. [Note: Phoenix produces 2 different kits to measure irisin, each having a different catalog number and different (intra-assay and inter-assay) detection limits. Thus comparisons of the results with different irisin kits can be invalid.