Metabolism Clinical and Experimental 87 (2018) 113–122

Contents lists available at ScienceDirect

Metabolism Clinical and Experimental

journal homepage: www.metabolismjournal.com

BDNF mimetic alleviates body weight gain in obese mice by enhancing mitochondrial biogenesis in skeletal muscle

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ARTICLE INFO

Article history:
Received 29 January 2018
Accepted 17 June 2018

Keywords:
7,8-Dihydroxyflavone
Obesity
Mitochondrial
Skeletal muscle

ABSTRACT

Background: 7,8-Dihydroxyflavone (7,8-DHF) is a small molecular weight compound that mimics the functions of brain-derived neurotrophic factor (BDNF). The current study aims to elucidate the molecular mechanism of 7,8-DHF-induced body weight regulation.
Methods: Obese female C57/BL6 (20-week-old) mice that have been fed with high-fat diet for 13 weeks were treated with 7,8-DHF for 9 weeks. Various biochemical and molecular analyses were performed to examine the signal transduction pathway, metabolite content, and mitochondrial mass in the animals. Moreover, systemic energy metabolism and insulin sensitivity were determined by indirect calorimetry and insulin/glucose-tolerance tests. We have also determined the metabolic actions of 7,8-DHF on cultured myotubes.
Results: 7,8-DHF treatment increased cellular respiration by promoting mitochondrial biogenesis in cultured skeletal muscle cells. In diet-induced obese mice, subsequent 7,8-DHF consumption triggered the AMPK/CREB/PGC-1α pathways to increase the muscular mitochondrial content. Systemic energy metabolism was thus elevated, which reduced the body weight gain in obese animals. Consequently, hyperlipidemia, hyperglycemia hyperinsulinemia, and ectopic lipid accumulation in skeletal muscle and liver of the obese animals were alleviated after 7,8-DHF treatment. Moreover, insulin sensitivity of the obese muscle was improved after 7,8-DHF consumption.
Conclusion: 7,8-DHF treatment increases muscular mitochondrial respiration and systemic energy expenditure, which alleviates the body weight gain and partially reverse the metabolic abnormalities induced by obesity.

1. Introduction

Mitochondrial behaviors including mitochondrial content, oxidative capacity, structural plasticity, and biogenesis are often impaired in obese tissues. [1–3]. As the central powerhouse and the major fatty acid oxidation (FAO) site in every cell, improper function of this vital organelle may disrupt the systemic energy homeostasis, which is a fundamental cause of obesity [4]. In support of this hypothesis, genetic manipulation studies in laboratory animals have shown a strong correlation between defective mitochondrial function and obesity development [5, 6]. On the other hand, interventions such as exercise and calorie restriction that possess beneficial metabolic effects are accompanied by an increased

Abbreviations: 7,8-DHF, 7,8-Dihydroxyflavone; ACC, accetyl CoA carboxylase; AdipoQ, adiponectin; AMPK, AMP-activated protein kinase; ANGPT-L3, angiopoietin-like 3; BAT, brown adipose tissue; BDNF, brain derived neurotrophic factor; CREB, cAMP responsive element binding protein; CRP, C-reactive protein; Cyto c, cytochrome c; DMH, dorsomedial hypothalamus; FAO, fatty acid oxidation; FFA, free fatty acid; HFD, high fat diet; HGF, hepatocyte growth factor; IGF1, insulin-like growth factor 1; IGFBP, insulin-like growth factor binding protein; IR, insulin receptor; iWAT, inguinal white adipose tissue; M-CSF, macrophage colony-stimulating factor; Nrf1, nuclear respiratory factor 1; PDH, pyruvate dehydrogenase; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1-α; PKC, protein kinase C; PLCγ, phospholipase C γ; PPARγ, peroxisome proliferator-activated receptor γ; RER, respiratory exchange ratio; SDHA, succinate dehydrogenase complex subunit A; Tfam, mitochondrial transcription factor a; TG, triglyceride; TIMP1, metallopeptidase inhibitor 1; TrkB, tropomyosin-related kinase receptor b; UCP1, uncoupling protein 1; VDAC, voltage-dependent ion channel; VEGF, vascular endothelial growth factor; VMH, ventral medial hypothalamus.

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https://doi.org/10.1016/j.metabol.2018.06.007
0026-0495/

mitochondrial content or improved mitochondrial activity [7, 8]. Based on these observations, restoring the mitochondrial number and function may represent a potential therapeutic strategy against obesity [9].

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family that plays an important role in synaptic plasticity, neuronal survival, neuron development and differentiation [10]. Through binding to its cognate receptor, the tropomyosin-related kinase receptor B (TrkB), BDNF triggers TrkB autophosphorylation, which leads to the activation of several signaling events including phosphoinositide 3kinase (PI3K)/Akt, Ras/extracellular signal-regulated kinase (ERK) and phospholipase C (PLCγ)/protein kinase C (PKC) pathways [11]. BDNF is also an anorexic factor that acts on the hypothalamic neurons [12, 13]. Because of its prominent role in suppressing food intake, manipulation of BDNF/TrkB signaling is a potential strategy in combating obesity development. However, the short half-life and non-bioavailable nature of BDNF prevents its clinical application [14]. A plant flavonoid, 7,8-dihydroxyflavone (7,8-DHF), has been identified as a functional BDNF mimic, which is able to induce TrkB receptor dimerization and activate its downstream signaling molecules [15]. Recently, we found that normal mice fed with high-fat diet (HFD) and 7,8-DHF simultaneously displayed reduced body weight gain via activating muscular TrkB [16]. Nevertheless, the study only proves that 7,8-DHF treatment plays a preventive role in the development of obesity; it remains unknown if 7,8-DHF has any therapeutic activity in mice that are already obese. In this study, we sought to examine if 7,8-DHF treatment could reverse the metabolic impairments induced by obesity as well as to further delineate the molecular mechanisms that bring about the anti-obesity activities of 7,8-DHF.

2. Methods

2.1. Chemicals and Reagents

C2C12 were purchased from ATCC (USA) and maintained as instructed. Differentiation of C2C12 was performed as reported [17]. 7,8-DHF was purchased from Tokyo Chemical Industry Co., Ltd. (Japan). In the cell culture experiment, 7,8-DHF was dissolved in DMSO to make a 1 M stock solution. The final concentration of DMSO in the culture medium was <0.1%. HFD (60% kcal) was obtained from Research Diet Inc. (USA). Other chemicals were purchased from Sigma-Aldrich (USA). Antibodies against pan Akt (#4691), pAkt S473 (#4060), pAMPKα T172 (#2535), AMPKα (#2603), pACC S79 (#3661), ACC (#3676), pERK T202/Y204 (#9106), ERK (#9102), pCREB S133 (#9198), CREB (#4820), PDH (#3205), Cycto c (#4280), VDAC (#4661), SDHA (#11998) and TrkB (#4603) were purchased from Cell Signaling (USA). Anti-pTrkB Y706 (ac135645) antibody was obtained from Santa Cruz Biotechnology (USA). Anti-UCP1 (ab155117) and PGC-1α (ab54481) antibodies were obtained from Abcam (USA). Anti-tubulin antibody (T6074) was obtained from Sigma-Aldrich (USA). IR phosphorylation ELISA kit was purchased from Cell Signaling (USA).

2.2. Animal Experiments

C57BL/6 J mice were obtained from the Jackson Laboratory (USA). Eight-week-old animals were used in our experiments. Mice were housed in environmentally controlled conditions with a 12-h light/dark cycle and had free access to food and water. Because 7,8-DHF administration only alleviated obesity development in female mice [16], all *in vivo* assays were thus done in 8-week-old female mice. The in vivo studies were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Oklahoma Health Sciences Center, and Committee on the Use of Live Animals in Teaching and Research (CULATR) of the University of Hong Kong. Euthanasia was performed by anesthetic overdose. Obesity was induced in C57BL/6 mice by feeding HFD that contains 20% kcal protein, 20% kcal carbohydrates and 60% kcal fat (lard) (D12492, Research Diet Inc., USA) for 3 months. The animals were then randomized to one of the two treatment groups. 7,8-DHF powder was dissolved directly in the drinking water to make a final concentration of 0.16 mg/ml. No organic solvent was used to enhance the solubility of the chemical. Unless indicated specifically, all tissue was collected from fed animals. Blood glucose level was measured by ACCU-CHEK Advantage Blood Glucose Meter (F. Hoffmann-La Roche Ltd., Switzerland). Serum insulin was measured by ELISA (Crystal Chem Inc., USA). Cholesterol, triglyceride and free fatty acid level in tissues or serum were measured using Cholesterol/Cholesteryl Ester Quantitation Colorimetric Kit, Triglyceride Quantification Colorimetric Kit and Free Fatty Acid Quantification Colorimetric Kit respectively (BioVision, USA). Serum TNFα was measured by ELISA (BD Biosciences, USA). Adipokine level in the blood was detected using the Proteome Profiler Mouse Adipokine Array Kit (R&D Systems, USA). Glucose tolerance test (GTT) was performed on mice after peritoneal injection of D-glucose (2 g/kg of body weight). Insulin tolerance test was performed on mice after peritoneal injection of recombinant insulin (0.75 U/kg Humulin, Eli Lily, USA).

2.3. Cell Culture and Fatty Acid Treatment

C2C12 myoblasts were cultured in DMEM with 5% FBS, 15% calf serum, 100 I.U./ml penicillin and 100 μg/ml streptomycin (Invitrogen, USA). Differentiation of myoblasts into myotubes was performed by incubating 100% confluent myoblast with differentiating medium (2% horse serum, 100 I.U./ml penicillin, and 100 μg/ml streptomycin) for 4 days. Differentiation of the C2C12 was confirmed by morphological changes as reported [17]. Insulin resistance of C2C12 myotubes was induced by stimulated the cells with 400 μM fatty acid-free BSA-coupled palmitic acid (PA) for 24 h as previously reported [18]. The PA-treated myotubes were then stimulated with 100 nM human insulin (Humulin, Eli Lilly, USA) for 30 mins, or 7,8-DHF (1 μM) for 24 h. Cell lysates were collected and used for Western blot analysis.

2.4. Western Blot Analysis

Tissues or cells were homogenized in lysis buffer containing 50 mM Tris, pH 7.4, 40 mM NaCl, 1 mM EDTA, 0.5% Triton X-100, 1.5 mMNa3VO4, 50 mM NaF, 10 mM sodium pyrophosphate, 10 mM sodium β-glycerophosphate, 1 mM phenylmethylsulfonyl fluoride, and 1× protease inhibitor cocktail (Sigma-Aldrich, USA). Cell debris was removed by centrifugation and the supernatant (cleared cell lysate) was collected for immunoblotting. Western blot results were visualized using Pierce ECL Western Blotting Substrate (Thermo Scientific, USA) and detected by the G:Box Chemi XRQ gel doc system (Syngene, USA).

2.5. RT- PCR

Total RNA from was prepared by Trizol Isolation Reagent (Invitrogen, USA). The first-strand cDNA from total RNA was synthesized using Superscript III reverse transcriptase (Invitrogen, USA) and Oligo-dT_{17} as the primer. Expressions of Tfam (F: CAGGAGGCAAAGGATGATTC, R: ATGCTC CGGATCGTTTCAC), Nrf1 (F: TCTCACCCTCCAAACCCAAC, R: ATGCTCTTG TACTTTCGCACCA) and β-Actin (F: AACCGTGAAAAGATGACCCAGAT, R: CACAGCCTGGATGGCTACGT) were detected using RealMasterMix SYBR ROX (5 Prime Inc., USA) on ABI7500 Real-time PCR System (Applied Biosystems, USA).

2.6. Determination of Mitochondrial Content, Mitochondrial Respiration and Lipid Oxidation

Total DNA was isolated by DNAeasy kit (Qiagen, USA). The amount of mitochondrial DNA was determined by real-time PCR using the primers 5′-CCCAGCTACTACCATCATTCAAGT-3′ (forward) and 5′-GATGGGTTTGG GAGATTGGTTGATGT-3′ (reverse) as reported [19]. The amount of β-actin gene in the genomic DNA was also examined (to normalize any variation due to the difference of total DNA extracted.