Free Radical Biology and Medicine 67 (2014) 396–407

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Hydroxytyrosol prevents diet-induced metabolic syndrome and attenuates mitochondrial abnormalities in obese mice

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ARTICLE INFO

Article history:
Received 3 September 2013
Received in revised form
26 November 2013
Accepted 27 November 2013
Available online 6 December 2013

Keywords:
Oxidative stress
Mitochondrial abnormalities
Insulin resistance
Hydroxytyrosol
Apoptosis
Free radicals

ABSTRACT

A Mediterranean diet rich in olive oil has profound influence on health outcomes including metabolic syndrome. However, the active compound and detailed mechanisms still remain unclear. Hydroxytyrosol (HT), a major polyphenolic compound in virgin olive oil, has received increased attention for its antioxidative activity and regulation of mitochondrial function. Here, we investigated whether HT is the active compound in olive oil exerting a protective effect against metabolic syndrome. In this study, we show that HT could prevent high-fat-diet (HFD)-induced obesity, hyperglycemia, hyperlipidemia, and insulin resistance in C57BL/6 J mice after 17 weeks supplementation. Within liver and skeletal muscle tissues, HT could decrease HFD-induced lipid deposits through inhibition of the SREBP-1c/FAS pathway, ameliorate HFD-induced oxidative stress by enhancing antioxidant enzyme activities, normalize expression of mitochondrial complex subunits and mitochondrial fission marker Drp1, and eventually inhibit apoptosis activation. Moreover, in muscle tissue, the levels of mitochondrial carbonyl protein were decreased and mitochondrial complex activities were significantly improved by HT supplementation. In db/db mice, HT significantly decreased fasting glucose, similar to metformin. Notably, HT decreased serum lipid, at which metformin failed. Also, HT was more effective at decreasing the oxidation levels of lipids and proteins in both liver and muscle tissue. Similar to the results in the HFD model, HT decreased muscle mitochondrial carbonyl protein levels and improved mitochondrial complex activities in db/db mice. Our study links the olive oil component HT to diabetes and metabolic disease through changes that are not limited to decreases in oxidative stress, suggesting a potential pharmaceutical or clinical use of HT in metabolic syndrome treatment.

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The metabolic syndrome (MS)[2] has been described as a cluster of abnormalities that includes a combination of obesity, dyslipidemia, impaired glucose tolerance, insulin resistance, and hypertension [1–3]. Although MS is becoming a major public health problem with increasing global prevalence, the underlying pathological mechanisms remain unclear. Among the known risk factors for MS, obesity is considered a central and causal risk factor [4,5]. Adipocyte abnormalities induce dysregulated production of cytokines, such as TNF-α, IL-6 [6], leptin [7], and adiponectin [8], which participate in the pathogenesis of obesity-associated MS.

Excessive fat accumulation is associated with systemic oxidative stress in humans [9]. Animal studies indicate that increased oxidative stress from fat accumulation is an early risk factor of MS [10]. Specifically, overproduction of reactive oxygen species (ROS) in the adipose tissue and liver of mice preceded the onset of obesity and insulin resistance induced by a high-fat diet (HFD) [11]. Additionally, Rector et al. [12] have demonstrated that hepatic mitochondrial dysfunction precedes the development of nonalcoholic fatty liver disease and insulin resistance in OLETF rats. Meanwhile, Vial et al. [13] reported that a HFD model results in both a decrease in the mitochondrial quinine pool and a profound modification in mitochondrial lipid composition, leading to inhibition of fatty acid oxidation and mitochondrial ROS production. An increasing number of studies have found a strong correlation between mitochondrial dysfunction induced by oxidative stress

Abbreviations: 4-HNE, 4-hydroxynonenal; CRP, C-reactive protein; FAS, fatty acid synthase; HDL-C, high-density lipoprotein cholesterol; HT, hydroxytyrosol; LDL-C, low-density lipoprotein cholesterol; MS, metabolic syndrome; SREBP-1c, sterol regulatory element-binding transcription factor 1c; TG, triglyceride

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http://dx.doi.org/10.1016/j.freeradbiomed.2013.11.029

and MS [14]. Because mitochondrial function is regulated by several processes, including mitochondrial biogenesis, dynamics, modification, and mitophagy [15–18], how mitochondrial dysfunction contributes to MS remains obscure.

Pharmacological therapy, such as metformin and thiazolidinedione, is helpful for treating MS, but is accompanied by several side effects [19,20]. Therefore, nutritional intervention, especially mitochondrial-targeted prevention, seems to be an efficient method for MS treatment. Dietary consumption of olive oil, an important component of the Mediterranean diet, is associated with a lowered incidence of cardiovascular disease. Additionally, there are a number of reports showing that olive leaf polyphenols, of which the major component is oleuropein, have beneficial effects on attenuating metabolic changes in MS [21] and improving insulin sensitivity [22]. However, the effective component of olive leaf extract and the underlying mechanisms of its action are still unclear. Hydroxytyrosol (HT), a natural polyphenol from virgin olive oil, is considered to be one of the most effective antioxidants [23,24]. Studies indicate that consumption of HT is associated with better heart health and anti-inflammation, with the underlying mechanism attributed to its ability to scavenge ROS and to enhance endogenous antioxidant systems [25,26]. In our previous study, we found that HT could protect retinal pigment epithelial (ARPE) cells against oxidative damage by activating the Nrf2/Keap1 pathway [27,28]. Moreover, HT could stimulate mitochondrial biogenesis in ARPE and 3T3-L1 cells [15,27] and could protect mitochondrial function in strenuous exercise-induced skeletal muscle fatigue [29]. These results implied that HT has beneficial effects on oxidative stress and mitochondrial function; therefore, we hypothesized that the beneficial effects of HT would occur in MS.

The most common murine models used for metabolic syndrome exhibit metabolic disorders induced by HFD or a nonfunctional leptin pathway, such as the db/db mouse [30]. The db/db mouse is a model of metabolic syndrome with obesity and type 2 diabetes but without hypertension. The leptin receptor activity is deficient in this kind of mouse because the mice are homozygous for a point mutation in the gene for the leptin receptor. In this study, both HFD-induced diabetic mice and db/db mice were used as MS models to test the beneficial effects and potential mechanisms of action of HT from olive oil.

Material and methods

Chemicals

Antibodies against β-actin were purchased from Sigma (St. Louis, MO, USA). Antibodies against NQO1, HO-1, SREBP-1c, Mfn1, and Mfn2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against glyceraldehyde-3-phosphate dehydrogenase (GAPDH), fatty acid synthase (FAS), Bcl-2, Bcl-X_L, Bak, Bax, and Bad were purchased from Cell Signaling Technology (Danvers, MA, USA). Antibodies against OPA1 and Drp1 were purchased from BD (Franklin Lakes, NJ, USA). Antibodies against complexes I, II, III, IV, and V were purchased from Invitrogen (Carlsbad, CA, USA). HT was purchased from APP-Chem Bio (Xi'an, China).

Animals and treatments

(1) For the HFD-induced diabetic mouse model, 4-week-old male C57BL/6 mice were purchased from the SLAC Laboratory Animal Co. Ltd. (Shanghai, China). After 1 week of acclimatization, the mice were randomly divided into four groups ($n=10$ in each group): mice fed a normal diet (control, 12% kcal fat content), mice fed a high-fat diet (HFD, 45% kcal fat content), mice fed a high-fat diet with a daily oral gavage of low-dose HT (10 mg/kg/day, HFD+HT 10), and mice fed a high-fat diet with a daily oral gavage of high-dose HT (50 mg/kg/day, HFD+HT 50). HT was prepared fresh in distilled water before gavage. After 17 weeks of feeding, the mice were fasted overnight and sacrificed.

(2) For the db/db diabetic mouse model, 4-week-old male db/db mice with the C57BL/6 J genetic background were purchased from SLAC Laboratory Animal Co. Ltd.. After 1 week of acclimatization, the mice were randomly divided into three groups: db/db, db/db mice with a daily oral gavage of HT (10 mg/kg/day), and db/db mice with a daily oral gavage of metformin (225 mg/kg/day). After 8 weeks of feeding, the mice were fasted overnight and sacrificed.

All animals were housed in a temperature- (22–28 °C) and humidity- (60%) controlled animal room and maintained on a 12-h light/12-h dark cycle (light from 0800 to 2000 hours) with food and water provided during the experiments. All of the procedures were performed in accordance with the U.S. Public Health Services *Guide for the Care and Use of Laboratory Animals,* and all efforts were made to minimize the suffering and the number of animals used in this study.

Oral glucose tolerance test

An oral glucose tolerance test (OGTT, 1 g/kg body wt) was performed after 17 weeks of feeding and gavage. All mice were fasted overnight before the OGTT. Blood was taken from the retrobulbar vein before and at 30, 60, 120, and 180 min after oral glucose gavage. The plasma glucose concentration was determined by the glucose oxidation method.

Sample preparation

After the mice were sacrificed, liver tissue and visceral fat pads including the perirenal and epididymal fat pads were removed and weighed. Blood samples were obtained by cardiac puncture, and the serum was separated by centrifugation (3000 rpm, 10 min). The levels of triglyceride (TG), total cholesterol (TC), LDL cholesterol, and HDL cholesterol were analyzed using an automated biochemistry analyzer (Hitachi Ltd., Tokyo, Japan). Serum levels of C-reactive protein (CRP), insulin, adiponectin, leptin, and IL-6 were measured using commercial kits according to the manufacturer's standards and protocols (RD Systems, Shanghai, China).

Histological analysis of liver samples

Liver tissue was excised, washed with ice-cold phosphate-buffered saline (PBS), and placed in 10% formalin. Several sections of tissue (thickness of 4–5 μm) were prepared and stained with hematoxylin and eosin (H&E) for histopathology and visualized by an Olympus BX71 microscope.

Isolation of skeletal muscle mitochondria

The gastrocnemius muscle was removed from each leg. One portion was frozen at −80 °C and used for extraction of total protein. The other portion was used immediately for isolation of mitochondria. The muscle was trimmed of fat and connective tissue and then chopped finely with a pair of scissors and used for mitochondrial isolation as previously described [17]. The mitochondrial protein concentration was determined using the BCA Protein Assay Kit (Pierce, No. 23225). Mitochondria were stored at −80 °C until enzyme analysis.

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