Nutrition, Metabolism & Cardiovascular Diseases (2014) **24**, 1144–1150

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

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

Chronic hydroxytyrosol feeding modulates glutathione-mediated oxido-reduction pathways in adipose tissue: A nutrigenomic study

E. Giordano, A. Dávalos, F. Visioli*

Laboratory of Functional Foods, Madrid Institute for Advanced Studies (IMDEA) – Food, CEI UAM+CSIC, Madrid, Spain

Received 17 February 2014; received in revised form 29 April 2014; accepted 11 May 2014
Available online 28 May 2014

KEYWORDS
Hydroxytyrosol;
Nutrigenomics;
Adipose tissue;
Mediterranean diet;
Cardiovascular disease

Abstract *Background and aim:* Hydroxytyrosol (HT) is the most prominent phenolic component of olives, olive oil, and their by-products, e.g. olive mill waste water. As the link between HT consumption (via extra virgin olive oil intake) and better cardiovascular prognosis is being scientifically validated, HT is entering the market as a potentially useful supplement for cardiovascular disease prevention. One of the target organs in cardiometabolic prevention is the adipose tissue, where inflammation, oxidative stress, and secretion of adipocytokines contribute to cardiovascular risk.
Methods and results: We explored the nutrigenomic effects of long-term supplementation with nutritionally-relevant doses of HT, i.e. 0.03 gm% – with specific reference to the adipose tissue and glutathione metabolism – and we explored underlying mechanisms in vitro. We show that HT modulates the antioxidant network in the adipose tissue, as mediated by glutathione (GSH) and associated enzymes. We also confirmed the GSH-modulating activities of HT in cultured adipocytes, where low, physiological HT concentrations were able to blunt the H_2O_2-induced GSH/GSSG alteration indicative of oxidative stress. In terms of surrogate markers of cardiovascular disease, we recorded significantly decreased circulating leptin concentrations in mice fed with HT as compared with controls.
Conclusions: HT – in nutritionally relevant amounts – is able to positively modulate the glutathione-driven antioxidant enzymatic machinery in the adipose tissue. Because HT is generally recognized as safe (GRAS) and exhibits an excellent safety profile in vitro and in vivo, its future employment as adjunct treatment of metabolic syndrome can be envisioned, pending specific trials.

Introduction

Hydroxytyrosol (HT) is the most prominent phenolic component of olives, olive oil, and their by-products such olive mill waste water (OMWW) [1]. Due to its diverse biological activities (many of which demonstrated in vivo), HT – either synthetic or derived from olive mill waste water and olive leaves – is being pharma-nutritionally exploited as a potential supplement or preservative [1]. Of note, most of the putatively healthful activities of HT have been demonstrated in the cardiovascular system; indeed, the European Food Safety Authority (EFSA) allows a health claim that recognizes olive oil phenolic compounds (including HT) as protectors of low-density lipoprotein (LDL) from oxidation [2]. Consequently, a link between HT consumption (via extra virgin olive oil intake) and better cardiovascular prognosis is being scientifically validated and HT is entering the market as a potentially useful supplement for cardiovascular disease prevention [1].

* Corresponding author. LabAFun, IMDEA – Food, Calle Faraday 7, 28049 Madrid, Spain. Tel.: +34 912796986.
E-mail addresses: francesco.visioli@imdea.org, fvisioli@yahoo.it (F. Visioli).

http://dx.doi.org/10.1016/j.numecd.2014.05.003
0939-4753/

The adipose tissue is not merely a storage depot for fat [3], but it plays important roles in cardiometabolism and its physio-pathology [4]. Notably, the adipose tissue secretes important hormones called adipocytokines [5], many of which are important regulators of body weight (leptin, adiponectin), of the immune system (TNF-α, IL-1, or IL-6), and of vascular function (angiotensin and PAI-1) [5]. A close link among obesity, a state of chronic low-level inflammation, and oxidative stress has been suggested [6]. It must be underscored that the multi-faceted interaction between inflammation, oxidants (electrophiles), and antioxidants (nucleophiles) is being slowly elucidated and that maintenance of "nucleophilic tone", by "para-hormesis", might provide useful means for regulating physiological nontoxic concentrations of the non-radical oxidant electrophiles that boost antioxidant enzymes and control inflammation [7]. In other words, most of the so-called dietary antioxidants actually work via para-hormesis and associated enzymatic network [7].

Even though the extent and precise nature of oxidative stress' contribution to cardiovascular disease has not been fully elucidated, the adipose tissue (be it central [8] or peri-vascular [9]) does contribute to cardiometabolic disorders via oxidation processes. In particular, the secretion of adipocytokines by the adipose tissue enhances systemic inflammation and exacerbates obesity-induced morbidities [6]. Accordingly, Codoñer-Franch et al. [6] proposed that decreasing the levels of chronic inflammation and oxidative stress may decrease cardiovascular morbidity and mortality, especially when interventions are performed early in life. In agreement with this hypothesis, glutathione (the most important low molecular weight, water soluble antioxidant) is inversely associated with body mass index [10]. Of note, an HT-containing OMWW preparation increased circulating glutathione concentrations in healthy volunteers [11].

In this study, we explored the nutrigenomic effects of long-term supplementation with nutritionally-relevant doses of HT – with specific reference to the adipose tissue and glutathione metabolism – and we explored underlying mechanisms in vitro.

Methods

Materials

Hydroxytyrosol was kindly donated by Seprox Biotech (Madrid, Spain). Hydrogen peroxide was from Sigma Aldrich (Madrid, Spain); Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), L-Glutamine, Penicillin–streptomycin and Trypsin–Versene (EDTA) were from Lonza Iberica (Barcelona, Spain); Insulin from porcine pancreas, 3-isobutyl-1-methylxanthine, HEPES Buffer solution 1 M in water were from Sigma Aldrich (Madrid, Spain); SuperScript III First-Strand Synthesis System for RT-PCR from Invitrogen; Qiazol from Qiagen (Izasa, Barcelona, Spain).

Animals and diets

This investigation conforms to the Guide for the Care and Use of Laboratory Animals, published by the US National Research Council (Eight Edition, 2010) and was approved by the Animal Experimentation Committee of the Universidad Complutense de Madrid.

Young C57BL/6 mice (2 months old, $n = 14$) were acclimatized and were kept on a 12:12 light/dark cycle, with the period of darkness between 7:00 PM and 7:00, for at least one week before the beginning of the experimentation. During this period, mice were fed a standard chow diet, and food and water were given *ad libitum*. After that, mice were maintained for eight weeks under two different diet regimens (Research Diets, Inc. New Brunswick, NJ, USA): 1) purified control diet ($n = 7$) or 2) purified control diet added with 0.03 gm% hydroxytyrosol ($n = 7$). This dose closely approximates human intake [1] and is a very low one once body surface area is taken into account [12]. Each diet provided 24.0%, 15.0%, and 61.0% kcal from protein, fat and carbohydrates, respectively and their detailed composition is given in Table 1. To reduce diurnal variations, animals were sacrificed between 10:00 and 11:00 a.m. Mice were anesthetized with isoflurane and dissected through a midline incision in the abdomen. Blood samples were collected from vena cava. Heparin (0.4 mg/ml) was injected by means of the iliac vein, and Hank's balanced salt solution (HBSS; pH 7.4) was perfused through the portal vein for 2 min to remove blood. The adipose tissue was quickly removed, washed twice in ice-cold HBSS, snap-frozen, and stored at −80 °C. In order to verify the effects of hydroxytyrosol on body weight and food intake, these parameters were periodically evaluated.

Determination of circulating leptin levels

All blood samples were drawn after an overnight fast and collected into centrifuge tubes containing the disodium salt of EDTA (1.5 mg/ml of blood). Plasma concentrations of leptin were determined by an ELISA kit, according to the manufacturer's instruction (Mouse Leptin, 96-well plate assay, Millipore, Madrid, Spain).

Cell culture

Murine 3T3-L1 pre-adipocytes (American Type Culture Collection, Barcelona, Spain) were cultured in proliferation medium (PM)

Table 1 Composition of the experimental diets.

	Control		Hydroxytyrosol	
	gm%	kcal%	gm%	kcal%
Protein	23	24	23	24
Carbohydrate	60	61	60	61
Fat	6	15	6	15
Ingredient	**gm/kg diet**			
Casein	244		244	
L-cystein	3		3	
Corn Starch	318		318	
Maltodextrin 10	45		45	
Dextrose	250		250	
Cellulose	75		75	
Inulin	25		25	
Sunflower oil	29.5		29.5	
Olive oil	18.6		18.6	
Lard	18.5		18.5	
Mineral mix S10026	10		10	
Dicalcium phosphate	13		13	
Calcium carbonate	5.5		16.5	
Potassium citrate	5.5		16.5	
Vitamin mix V10001	10		10	
Retinyl acetate, 500,000 IU/gm	0.048		0.048	
Choline bitartrate	2		2	
Hydroxytyrosol	0		0.3	
Cholesterol	0.146		0.146	
Total	**1083.84**		**1084.14**	