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Effects of dietary extra-virgin olive oil on oxidative stress resulting from exhaustive exercise in rat skeletal muscle: A morphological study

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ABSTRACT

Physical exercise induces oxidative stress through production of reactive oxygen species and can cause damage to muscle tissue. Oxidative stress, resulting from exhaustive exercise is high and improvement of antioxidant defenses of the body may ameliorate damage caused by free radicals. Extra-virgin olive oil is widely considered to possess anti-oxidative properties. The aim of this study was to determine if extra-virgin olive oil improved the adaptive responses in conditions of oxidative stress. Twenty-four 12-week-old male Sprague-Dawley rats were divided in three groups: (1) rats fed with standard chow and not subjected to physical exercise; (2) rats fed with standard chow and subjected to exhaustive exercise; (3) rats fed with a diet rich in oleic acid, the major component of extra-virgin olive oil, and subjected to exhaustive exercise. Exhaustive exercise consisted of forced running in a five-lane 10° inclined treadmill at a speed of 30 m/min for 70–75 min. We studied some biomarkers of oxidative stress and of antioxidant defenses, histology and ultrastructure of the *Quadriceps femoris* muscle (*Rectus femoris*). We observed that, in rats of group 3, parameters indicating oxidative stress such as hydroperoxides and thiobarbituric acid-reactive substances decreased, parameters indicating antioxidant defenses of the body such as non-enzymatic antioxidant capacity and Hsp70 expression increased, and *R. femoris* muscle did not show histological and ultrastructural alterations. Results of this study support the view that extra-virgin olive oil can improve the adaptive response of the body in conditions of oxidative stress.

Introduction

The Mediterranean diet is identified as the traditional dietary pattern found in the Mediterranean areas of Crete, Greece, and southern Italy in the late 1950s and early 1960s (Willett et al., 1995). The Mediterranean diet consists of a high consumption of whole grains, legumes, nuts, vegetables, and fruits with a relatively high fat consumption (up to 40% of total energy intake), mostly from monounsaturated fatty acids (MUFA; up to 20% of energy) mainly provided by olive oil (OO), the principal source of culinary and dressing fat. The Mediterranean diet includes moderate to high consumption of fish, with poultry and dairy products (usually yogurt or cheese) consumed in moderate to small amounts. In addition there is low consumption of red meats, processed meats and meat products; and moderate alcohol intake, usually in the form of red wine consumed with meals. This dietary pattern was represented by a food pyramid (Willett et al., 1995). OO is the principal fat and one of the cornerstones of the Mediterranean diet. The beneficial effects of OO may be due to its components such as phenolic compounds, tocopherol, carotenoids, which have been shown to possess antimicrobial, antioxidant and anti-inflammatory properties (Cicerale et al., 2012). Phenolic compounds in OO exert beneficial effects on lipid oxidation, deoxyribonucleic acid (DNA) oxidative damage and in general oxidative stress, *in vitro* and *in vivo*, with subsequent positive effects on disease risk. This interaction is primarily related to their ability to scavenge free radicals, thus preventing cellular injury. An excess of free radicals can cause oxidative damage to biomolecules (i.e. lipids and DNA), increasing the risk of developing various chronic diseases such as atherosclerosis, cardiovascular disease, cancer, chronic inflammation, stroke and other degenerative diseases.

Reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical and singlet oxygen, are free radicals in which oxygen is involved. ROS often cause cellular alterations, depending on the nature of the ROS. Production of ROS is a cellular process that during physical exercise is indicative of oxidative stress, in association with the increased oxygen intake during intensive

Abbreviations: BPs, olive biophenols; DNA, deoxyribonucleic acid; H&E, hematoxylin and eosin; HSPs, heat shock proteins; MAC, Mediterranean alimentary culture; MUFA, monounsaturated fatty acid; NEAC, non-enzymatic antioxidant capacity; OO, olive oil; ROS, reactive oxygen species; TBARS, thiobarbituric acid-reactive substances; TEM, transmission electron microscope.

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or endurance muscular performance (Castrogiovanni and Imbesi, 2012). It is widely reported that during physical exercise free radicals, including ROS, remove electrons from lipid membranes, resulting in a chain of reactions called "lipid peroxidation" and its extent depends on the intensity of exercise (Preta et al., 2010). If the amount of free radicals is excessive, increased oxidative stress can damage muscle tissue, inhibit performance and induce fatigue (Schneider et al., 2005). During muscular contraction, superoxide and hydrogen peroxide, not particularly harmful ROS, are produced in muscle cells and then released in the extracellular matrix where they contribute to production of more dangerous hydroxyl radicals (Reid, 2008). It has been observed that hydroxyl radicals, powerful oxidants, are mostly generated during hard muscular work (Diaz et al., 1993). If physical exercise causes an increase in ROS production, aerobic physical activity also promotes specific adaptations in relation to type, intensity and duration of the physical exercise performed. For example, one of these cytoprotective responses in skeletal muscle is an increased production of heat shock proteins (HSPs) that provide a rapid recovery and tissue remodeling when damage occurs after subsequent periods of oxidative stress (Broome et al., 2006). HSPs help preserve cellular integrity through several mechanisms including the ability to repair many proteins (Kayani et al., 2010). After physical exercise, in the recovery phase, antioxidant defense processes of the body can regulate the amounts of free radicals (Blokhina and Fagerstedt, 2010).

In addition to physical activity, diet is another parameter that needs to be considered, as exogenous dietary antioxidants interact with endogenous antioxidants (Ochoa et al., 2003). Published data related to supplementation of exogenous antioxidants remain controversial, and it is still unclear if any damage of regular and vigorous exercise will be ameliorated by antioxidant dietary supplementation (Ozkanlar and Akcay, 2012). Many nutrients can exert anti-oxidative activity in humans, and one of these may be extra-virgin olive oil, typical of the Mediterranean diet. Extra-virgin olive oil is rich in biophenols (BPs), such as phenolic acids, hydroxytyrosol, oleuropein whose metabolic role is fundamental in human nutrition as antioxidants and defenders against cancer, HIV, inflammation, virus and bacteria (Sivakumar and Uccella, 2010), and oleic acid (monounsaturated fatty acid, MUFA). Several studies support the idea that the antioxidant role of extra-virgin olive oil is due mainly to its high content in oleic acid, up to 78% (Ochoa et al., 2003; Perez-Martinez et al., 2011). In our study we evaluated the effects of extra-virgin olive oil in the daily diet of rats submitted to exhaustive exercise, a cause of oxidative stress. In particular we evaluated some biomarkers of oxidative stress, such as hydroperoxides and thiobarbituric acid-reactive substances (TBARS), and some of the adaptive responses of the body, such as non-enzymatic antioxidant capacity (NEAC) and heat shock protein 70 (Hsp70). We also investigated if any alteration in the skeletal muscle tissue occurred. The aim of our study was to verify if a diet rich in oleic acid, such as extra-virgin olive oil, improved the adaptive response of the body in conditions of oxidative stress due to physical effort.

Materials and methods

Breeding and housing of animals

In our study, we used twenty-four 12-week-old healthy male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, USA), with an average body weight of 200 ± 40 g. Rats were individually housed in polycarbonate cages (cage dimensions: $10.25''W \times 18.75''D \times 8''H$) during the entire period of the study and were housed at controlled temperature (20–23 °C) and humidity, with free access to water and food and photoperiod of 12 h light/dark. All efforts were made to reduce the number of animals used and to minimize animal suffering. The experimental procedures were performed in accordance with the European Community Council Directive (86/609/EEC) and Italian Animal Protection Law (116/1992).

Table 1
Composition of experimental and standard chow (g/100 g).

Casein	27.7
Starch	7.53
Sucrose	31.36
Vitamin mixture	1.0
Mineral mixture	2.68
Cellulose	0.84
Choline	0.09
Methionine	0.3
Lipids	28.5

Experimental design

A total of 24 rats were divided into three groups ($n = 8$) and housed individually in polycarbonate cages. Group 1: rats fed *ad libitum* with standard rat chow containing carbohydrates (40%), proteins containing all essential amino acids (30%), and lipids (30%). Lipids were a mixture of neutral fatty acids, saturated fatty acids and unsaturated fatty acids. Rats were permitted free cage activity without joint immobilization and they were not subjected to physical exercise. Group 2: rats fed *ad libitum* with standard rat chow containing carbohydrates (40%), proteins containing all essential amino acids (30%), and lipids (30%). Lipids were a mixture of neutral fatty acids, saturated fatty acids and unsaturated fatty acids. Rats were subjected to exhaustive exercise (see "Exercise protocol"). Group 3: rats fed *ad libitum*, with experimental rat chow containing carbohydrates (40%), proteins containing all essential amino acids (30%), and vegetable origin lipids (30%). The composition of standard and experimental chow was identical except for lipids, in where the experimental diet lipids consisted of extra-virgin olive oil, rich in oleic acid (74–77%) (MUFA) (Tables 1 and 2). Rats were subjected to exhaustive exercise (see "Exercise protocol").

Throughout the experiment there were no significant differences in the mean food consumption, (about 20 g per day per rat) nor in the body weights of the animals in the three groups.

Immediately after exhaustive exercise, rats were sacrificed by intracardial Pentothal® injection 30–40 mg/kg (Biochemie, Kundl, Austria); under Furane 2% ®-narcosis (Abbott Laboratories, Maidenhead, Berks., UK). *Quadriceps rectus femoris* muscles from both thighs and liver samples were collected from rats for a total of 24 samples. The samples were used for histological and ultrastructural observations and for Hsp70 evaluation by immunohistochemistry and Western blot. Prior to sacrifice blood was collected from the three groups of rats. Under light ether narcosis, 1.5 ml of blood was withdrawn from the jugular vein into syringes preloaded with 200 or 500 μl of 3.13% trisodium citrate anticoagulant. Plasma was used to measure hydroperoxides, TBARS and NEAC.

The Sicilian Association of Olive Cultivators provided us with the extra-virgin olive oil (year 2011–2012) derived exclusively from *Olea europea sativa*. Oil was obtained exclusively by mechanical pressing of olives under controlled conditions, especially

Table 2
Fatty acid composition of (%) of experimental and standard chow.

	Experimental	Standard
Palmitoleic acid	2.4	10.3
Palmitic acid	7.8	25.8
Stearic acid	1.9	20.7
Oleic acid	76.7	29.9
Linoleic acid	7.7	12.4
Linolenic acid	3.5	1.8

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