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Applied nutritional investigation

Effect of 1-y oral supplementation with vitaminized olive oil on platelets from healthy postmenopausal women

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ABSTRACT

Objective: Olive oil is the main fat source in the Mediterranean diet and shows a protective role against aging and related diseases. Osteoporosis represents a serious health problem worldwide and is associated with an increased risk for fractures and mortality. Nutrition should be part of bone disease prevention strategies, especially in light of the aging population and the effect of diet on bone health. The aim of this study was to investigate whether oral supplementation with extra virgin olive oil (VOO) enriched with vitamins D_3, K_1, and B_6 (VitVOO) is able to modify some physicochemical and functional plasma membrane properties and nitrosative stress markers status.

Methods: In this single-center, randomized placebo-controlled trial, 60 postmenopausal women were administered either VitVOO or placebo (PlaVOO). After 1 y of oral supplementation, platelet membrane fluidity changes, Na^+/K^+-ATPase activity, serum nitric oxide, and peroxynitrite levels were determined in participants.

Results: After 1 y (time 1), women taking VitVOO showed lower nitric oxide levels than those taking PlaVOO; the same trend was found for peroxynitrite levels. As far as membrane fluidity was concerned, a significant decrease in anisotropy of diphenylhexatriene and trimethylammonium-diphenylhexatriene at time 1 in VitVOO participants compared with PlaVOO was found. Finally, Na^+/K^+-ATPase activity showed a significant increase after VitVOO supplementation.

Conclusion: The supplementation of VitVOO into the diet of postmenopausal women could represent a proper tool for platelet function and a useful strategy against nitrosative stress and related diseases, thus confirming the antioxidant role played by the added vitamins.

Introduction

Olive oil (OO) is an integral ingredient of the Mediterranean diet, and accumulating evidence suggests that it may have healthy benefits in terms of reduction of cardiovascular risk factors. In fact, virgin olive oil (VOO) plays a pivotal role as the main source of fat in the Mediterranean diet, a diet that has traditionally been linked to longevity in Mediterranean populations and is associated with a significant improvement in health status, as measured by reduced mortality from several chronic diseases. More than 90% of OO is formed in the mesocarp of the drupe from the fruit of the olive tree (*Olea europaea L.*) [1–8].

The cardioprotective effects of OO have been ascribed to its content of monounsaturated fatty acids (MUFAs) such as oleic acid and the presence of other biologically minor constituents such as polyphenols, tocopherols, and triterpenoids [1,9].

In vitro and ex vivo models have shown different polyphenol properties as antioxidant, as preventive of DNA oxidation and endothelial dysfunction by quenching free radicals, inhibitor of platelet aggregation, and antiinflammatory activity [10]. Some of these properties, such as antioxidants and antithrombotics, have the capacity to improve endothelial function and are reproduced in humans as well and revised elsewhere [11,12].

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Menopause, the permanent cessation of menstrual cycles, which occurs between the ages of 45 and 55 y, signals the end of the fertile phase of a woman's life. It is characterized by an estrogen-deficient state that is known to increase the risk for cardiovascular diseases (CVDs) [13]. Lipid changes that accompany menopause account for only few cases of coronary artery disease [14].

The loss of endothelial nitric oxide (NO) bioavailability is a key mechanism underlying endothelial dysfunction, typical of cardiovascular injuries, and increased expression of adhesion molecules and chemoattractants. NO bioavailability is reduced either by decreased formation or by enhanced removal of NO. The presence of classical cardiovascular risk factors is associated with enhanced generation of radical oxygen species where superoxide anions (O_2^-) play a pivotal role by reacting with NO resulting in the formation of peroxynitrite ($ONOO^-$), and hence decreasing bioavailability of NO [15,16]. Such reductions may impair the osteoblast–osteoclast balance with unpredictable consequences on bone remodeling [17]. It has been shown that NO has significant modulatory effects on bone metabolism [18]. NO is produced constitutively by osteoblasts [19] and stimulates their proliferation in vitro [20].

It also has been shown that NO bioactivity is reduced in postmenopausal women, and low serum levels of NO are suspected as a risk factor [21]. CVD incidence markedly increases after menopause, becoming the leading cause of morbidity and mortality in postmenopausal women [22].

The aim of the present study was threefold:

1. Confirm whether 1 y of oral supplementation with either 20 mL/d of extra virgin olive oil (VOO) enriched with vitamins D_3 (1,25-dihydroxy-cholecalciferol), K_1 (phylloquinone) and B_6 (pyridoxal 5′-phospate) (VitVOO) or 20 mL/d of VOO used as placebo (PlaVOO), was able to decrease NO and high-reactive oxygen species (hROS) levels in platelets before and after such intake. Interesting results were observed in fertile women (data not published).
2. Verify any possible modification of the physicochemical and functional properties of platelets by means of membrane fluidity and Na^+/K^+-ATPase activity.
3. Attest any possible correlations among the aforementioned markers.

Concerning the use of platelets, a relationship between platelet functions, platelet originating factors, and bone mineral density has been demonstrated [23].

For its characteristics, VOO is a functional food and can be considered a good means for the absorption of beneficial nutrients maintaining them intact; thus, through a fortification process the oil can be enriched by adding nonenergetic nutrients (i.e., vitamins, minerals, or both) without modifying the oil energy content. Thus, VOO can be used in the diet when conditions of nutrition deficiency are present.

Methods

Design overview

The present study was performed in accordance with the Declaration of Helsinki as revised in 2001 and was approved by the Institutional Review Board of Senigallia Hospital. Written informed consent was provided by all participants after the procedures were fully explained.

This study was designed as a 1-y single-center, randomized placebo-controlled trial of daily oral supplementation of 20 mL/d of VOO containing vitamin K_1 (0.70 mg/100 mL), vitamin D_3 (50 mg/100 mL), and vitamin B_6 (6 mg/100 mL) versus placebo (20 mL/d of PlaVOO) bottled in unlabeled containers.

Table 1
Composition of the tested oils: PlaVOO and VitVOO enriched with vitamin K_1 (0.70 mg/100 mL), vitamin D_3 (50 μg/100 mL), and vitamin B_6 (6.0 mg/100 mL)

	PlaVOO (per 100 mL)	VitVOO (per 100 mL)	PlaVOO (% RDA)	VitVOO (% RDA)
Energy value, kcal	837	828	–	–
Proteins, g	0	0	–	–
Carbohydrates, g	0	0		–
Fats, g	93.3	92	–	–
Saturated, g	13.3	13.8		
Monounsaturated, g	66.6	69		
Polyunsaturated, g	13.3	9.20		
Vitamin D_3, μg	0	50	–	100
Vitamin K_1, mg	0	0.70	–	100
Vitamin B_6, mg	0	6	–	30
Vitamin E (natural), mg	30	30	30	30

B_6, pyridoxal 5′-phospate; K_1, phylloquinone; PlaVOO, vitaminized placebo virgin olive; RDA, Recommended Daily Allowance; VitVOO, vitaminized virgin olive oil.

The oil formulation was prepared by Fattoria Petrini (Monte San Vito, Ancona, Italy). The composition of tested oils is shown in Table 1.

Study participants and intervention protocol

Sixty white postmenopausal women (ages 50–61 y), enrolled at a postmenopausal ambulatory center of the Department of Obstetrics, Gynecology and Pediatrics, Senigallia Hospital (Ancona, Italy) for health screening for osteoporosis, were randomly assigned to the two groups.

Women were eligible to participate in the study if they were postmenopausal (≥1 y after last menses). None of the women had decompensated diseases of the liver, kidney, pancreas, lung, or heart; a history of active cancer in the past 5 y; chronic disease (e.g., diabetes mellitus, CVD, cancer, or fat malabsorption syndromes), abnormal bleeding, or thrombotic disorders. Women regularly taking nonsteroidal antiinflammatory drugs or drugs known to alter platelet function or the hemostatic system in general were excluded from the study. Furthermore, women were asked not to take any such medications for ≥2 wk before beginning of the study and 2 wk before the final follow-up visit.

The selected women were required to have normal screening laboratory values, were nonsmokers, nonalcoholics, and had a body mass index (BMI) in the range of 22 to 28 kg/m^2. The women consumed a standard Mediterranean diet (based on a list providing information on permitted food), that was balanced for each enrolled individual and consisted of 1500 kcal/d; 15% proteins, 30% lipids, 55% carbohydrates, and 34 g of fiber, prescribed 2 wk before the beginning of the study. They were instructed to avoid taking dietary supplements other than those allowed through the study and food sources of vitamin K such as green vegetables (i.e., spinach, broccoli, green beans, asparagus) and fermented foods, like cheese. They were educated to use the oil only as a dressing, but not for cooking. All dietary recommendations were performed by a physician on an individual basis. Dietitians took care of providing correct and useful instruction for day-to-day management and food preparation, and meetings were scheduled on a monthly basis to verify that patients were correctly following the diet recommended and that they did not change dietary supplementation. Baseline measurements (T_0) were performed in January 2011, and follow-up measurement (T_1) was performed after 1 y. On each occasion, blood samples were drawn between 0700 and 1000 after overnight fast.

At each meeting, leftover oils were counted to assess compliance. Participants taking ≥95% of the prescribed oil were considered compliant.

Blood sampling

Blood was collected into vacutainers containing citrate dextrose (36 mL citric acid, 5 mM KCl, 90 mM NaCl, 5 mM glucose, 10 mM EDTA, pH 6.8). Samples for routine laboratory measurements were processed immediately.

Blood was collected on ice and centrifuged at 200*g* for 10 min at 4°C within 2 h from withdrawal, and the middle layer of the platelet-rich plasma (PRP) was rapidly pipetted off and used to obtain platelets.

Platelet isolation

Platelets were isolated by differential centrifugation in antiaggregation buffer (Tris-HCl 10, NaCl 150, EDTA 1, and glucose 5 mm; pH 7.4) according to Vignini et al. [24]. PRP was washed three times in antiaggregation buffer and centrifuged as above to remove any residual erythrocytes. A final centrifugation at 2000*g* for 20 min was performed to isolate the platelets. The platelet pellet was