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A Mediterranean-style low-glycemic-load diet increases plasma carotenoids and decreases LDL oxidation in women with metabolic syndrome☆

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Abstract

Thirty-five women with metabolic syndrome and high plasma low-density lipoprotein (LDL) cholesterol (≥100 mg/dl) participated in a dietary intervention consisting of a Mediterranean-style low-glycemic-load diet for 12 weeks. Participants were randomly allocated to consume diet only (n=15) or diet plus a medical food containing soy protein and plant sterols (n=20). Plasma concentrations of carotenoids, lipoprotein subfractions and oxidized LDL (OxLDL) were measured. Independent of treatment, women had a significant increase in plasma lutein (P<.0001) and β-carotene (P<.0001), while plasma lycopene was reduced (P<.05) after 12 weeks. Low-density lipoprotein cholesterol was reduced from 138±35 to 114±33 mg/dl (P<.0001). In addition, decreases were observed in the atherogenic subfractions: large very low-density lipoprotein (P<.05), small LDL (P<.00001) and medium high-density lipoprotein (P<.05). Oxidized LDL was significantly reduced by 12% in both groups (P<.01). Changes in OxLDL were inversely correlated with plasma lutein (r=-.478, P<.0001). The data indicate that women complied with the dietary regimen by increasing fruits and vegetable intake. Decreased consumption of high-glycemic foods frequently co-consumed with lycopene-rich tomato sauce such as pasta and pizza may be responsible for the lowering of this carotenoid in plasma after 12 weeks. These results also suggest that plasma lutein concentrations may protect against oxidative stress by reducing the concentrations of OxLDL.

Keywords: Mediterranean diet; Plasma carotenoids; Oxidized LDL; Metabolic syndrome

1. Introduction

Metabolic syndrome (MetS) is a cluster of metabolic alterations, including central obesity, hyperglycemia, low high-density lipoprotein cholesterol (HDL-C) concentrations, hypertension and hypertriglyceridemia. MetS is associated with a high risk of developing cardiovascular disease (CVD), type 2 diabetes and all-cause mortality [1]. A lower prevalence of MetS has been associated with several components of the Mediterranean diet pattern, which has long been linked with greater longevity and reduced mortality and morbidity for coronary heart disease (CHD), certain cancers and other nutrition-related diseases [1–3].

An important step toward elucidating diet and CVD risk relationships in humans is the development of techniques for monitoring and characterizing dietary exposure [4]. Usually, this is accomplished by using dietary assessment instruments such as 24-h dietary recalls, food record, and food frequency questionnaires [5]. However, these tools are subjective and are prone to reporting errors [5]. A biomarker is less susceptible to the inherent errors associated with traditional diet assessment methods, may be less subject to intraindividual variation and can allow assessment of compliance in dietary intervention trials where a true placebo arm is not feasible [4].

Vegetables and fruits are key components of a Mediterranean diet [4,6–8]and represent the main sources of carotenoids in the human diet [9]. Consequently, plasma carotenoid levels have been found to be useful biomarkers of vegetable and fruit intake. Of the more than 700 naturally occurring carotenoids identified to date, six (β-carotene, β-cryptoxanthin, α-carotene, lycopene, lutein and zeaxanthin) are commonly found in blood (>95% total blood carotenoids) [10]. Carotenoids have potential antioxidant properties due to their chemical structure with abundant conjugated double bonds that interact with cellular membranes because of their fat-soluble nature [11]. The

Abbreviations: BHT, butylated hydroxytoluene; CHD, coronary heart disease; CVD, cardiovascular disease; HDL-C, HDL cholesterol; HEAT, hexane/ethanol/acetone/toluene, 10:6:7:7; HPLC, high-performance liquid chromatography; LDL-C, LDL cholesterol; MetS, metabolic syndrome; MTBE, methyl *tert*-butyl ether; NMR, nuclear magnetic resonance; OxLDL, oxidize LDL.

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majority of circulating carotenoids are associated with lipoproteins, but predominantly with low-density lipoprotein (LDL), the major cholesterol-transporting lipoprotein [12].

Oxidative modification of LDL (OxLDL) is a key event in the oxidation hypothesis of atherogenesis and is suggested to increase the risk of CVD [13]. The ability of LDL to resist oxidation is influenced by several endogenous factors, among which the content of tocopherols and carotenoids are prominent [14]. It has been reported that particle contents of lutein/zeaxanthin, β-cryptoxanthin, β-carotene and lycopene were markedly reduced in small, dense LDL [14], a particle that is known to be more prone to oxidation.

Studies have shown inconclusive results regarding the ability of carotenoids to affect oxidation of LDL. For example, a high dietary intake of tomato products increased the resistance of LDL to oxidation in healthy normocholesterolemic adults [15]. Likewise, in diabetic patients, the increased susceptibility to LDL oxidation was normalized by β-carotene dietary supplementation [16]. In a subgroup of subjects from the Prevención con Dieta Mediterránea (PREDIMED) study, which evaluated the effects of this dietary pattern in patients on primary cardiovascular prevention, OxLDL levels decreased significantly after 3 months of consuming a Mediterranean diet [17]. However, a human study showed that supplementation with either a carotene mixture or lycopene had no effect *in vitro* on LDL oxidation, despite significant increases in plasma and LDL concentrations of lycopene, α-carotene and β-carotene [18].

Some studies have demonstrated that supplementation with eggs, as a source of dietary carotenoids, positively modulates plasma carotenoid and lipoprotein subclasses [19,20]. However, to the best of our knowledge, there is no information about the effect of a Mediterranean-style low-glycemic-load diet on OxLDL levels in women with MetS.

The aims of this study were (1) to measure the effects of a Mediterranean-style low-glycemic-load diet alone or in combination with a medical food containing soy protein, vitamins and plant sterols, on plasma carotenoid levels, lipoprotein subfractions and size and plasma concentrations of OxLDL; (2) to establish relationships between dietary intake of lutein, zeaxanthin, β-carotene and lycopene and their plasma concentrations in order to assess adherence to the diet; and (3) to determine associations between plasma carotenoid concentrations and OxLDL levels and lipoproteins subfractions. Our hypothesis was that the consumption of a Mediterranean-style diet would increase plasma carotenoid concentrations and that increases in circulating carotenoids would be associated with decreases in both OxLDL levels and atherogenic lipoproteins in women with MetS.

2. Experimental procedure

2.1. Materials

Lutein and zeaxanthin were purchased from Chromadex (Irvine, CA). β-Carotene was purchased from Sigma-Aldrich (St. Louis, MO). Pure lycopene was isolated and crystallized from tomato paste as previously described [21]. All solvents were high-performance liquid chromatography (HPLC) grade and were purchased from Fisher Scientific (Pittsburgh, PA). Butylated hydroxytoluene (BHT) was also purchased from Fisher Scientific. The medical food was provided by Metagenics (Gig Harbor, WA). Oxidized LDL kits were purchased from Alpco (Salem, NH).

2.2. Experimental design

We initially recruited 39 women with MetS and high plasma concentrations of LDL cholesterol (LDL-C ≥100 mg/dl). Subjects were randomly assigned to consume either a Mediterranean-style low-glycemic-load diet alone (diet-only group; $n=19$) or the same diet plus a medical food containing soy protein, plant sterols, rho *iso*-alpha acids from hops and proanthocyanidins from the acacia tree (medical food group; $n=20$). The composition of the medical food is presented in Table 1. Women were asked to follow their assigned diet for 12 weeks without changes in their physical activity level. All experimental protocols were approved by the University of Connecticut Institutional Review Board, and informed consents were obtained from all subjects. Four subjects from the diet-only group did not finish the study for several reasons, including difficulty in following the diet or for other personal reasons. Thus, only 15 women from the diet-only group completed the study. Plasma samples were obtained at baseline and Week 12 to measure plasma carotenoids, lipids, lipoprotein subfractions and size, and OxLDL.

2.3. Dietary records

For the assessment of compliance, subjects provided 3-day dietary records every 2 weeks. Records included two weekdays and one weekend. Nutrient intake was evaluated using Nutritional Data Systems software Version 8.0, developed by the Nutrition Coordinating Center, University of Minnesota (Food and Nutrient Database 29, Minneapolis, MN).

2.4. Blood handling

Following a 12-h fast, whole blood was collected into tubes containing 0.10 g/100 g EDTA. Plasma was separated by centrifugation at 1500×*g* for 20 min at 4°C and placed into vials containing phenyl methyl sulfonyl fluoride (0.01 g/100 g), sodium azide (0.01 g/100 g) and aprotinin (0.05 g/100 g). Plasma samples were aliquoted and stored at −80°C for further analysis.

2.5. Carotenoid Extraction

Plasma (0.5 ml) was mixed with ethanol containing 0.1% BHT (0.5 ml) and 2.5 ml of HEAT [hexane/ethanol/acetone/toluene (HEAT), 10:6:7:7, v/v/v/v]. Samples were vortexed and centrifuged for 10 min at 300×*g*. The upper layer (containing carotenoids) was collected, and the remaining aqueous plasma mixture was extracted once more with 2.5 ml HEAT. The extracts were pooled and dried immediately under N_2 gas. Dried extracts were stored at −80°C for no more than 24 h before HPLC analysis. All experiments were performed under red light.

Table 1
Macronutrient, vitamins, isoflavones and plant sterol content of the medical food

Nutrient	Amount per day
Energy (kJ)	1394
Total fat (g)	6
Protein (g)	30
Total carbohydrate (g)	48
Dietary fiber (g)	8
Retinyl palmitate (IU)	3500
Ascorbic acid (mg)	120
Cholecalciferol (IU)	80
α-Tocopheryl acetate (IU)	22
Thiamin (mg)	1.5
Riboflavin (mg)	1.7
Niacin (mg)	20
Vitamin B6 (mg)	50
Folate (μg)	800
Vitamin B12 (μg)	60
Biotin (μg)	300
Pantothenic acid (mg)	10
Isoflavones (mg)	34
Plant sterols (mg)	4000