PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 9 (1): 15-26, 2010
ISSN 1680-5194

Blood Lipid Profile, Oxidation and Pressure of Men and Women Consumed Olive Oil

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Abstract: Hypercholesterolemia and hypertension is one of the most important risk factors for Coronary Heart Disease (CHD). Recent studies have pointed out the possibility that olive oil may reduce these factors. The present study was designed to assess the effect of three olive oils contained different levels of phenolic comounds on blood lipid profile, oxidation status and pressure of normo cholesterol and pressure men and women. 12 men and 13 women participated in the study. Subjects consumed there habitual diets with low phenol-, medium phenol-, or high phenol- containing olive oil for 4 weeks each with a 4-week washout period between them. Consumption of these oils was nonsignificantly reduced triglycerides, total cholesterol, free cholesterol and cholesterol ester concentrations and no marked effect in phospholipids concentration. However, resulted in significantly reduction in LDL-c and rising in HDL-c concentrations in plasma of men and women. These reduction and rising effects were increased with increasing the phenols content and no worth differences between men and women. Plasma contents of α-tocopherol, β-carotene and retinol were nonsignificantly and squalene and phenol were significantly increased after consumption of olive oils in both sexes compared with the base lines. Plasma malondialdehyde level and blood pressure (systolic and diastolic) were significantly reduced with increasing phenol content in consumed oil. In conclusion, dietary olive oil with high phenol content proved to be helpful in reducing the CHD risk factors and normalize blood pressure systolic pressure of men and women.

Key words: Olive oils, minor components, phenols, TC, LDL-c, HDL-c, MDA, BP

INTRODUCTION

Olive oil production in Jordan is increasing from year to year with increasing growing areas. Jordan's annual production of olive oil is estimated at 32,000 tons. Roughly, Jordan's five million people consume about 22.5 million kg of olive oil every year (4.5 kg/person). As in diets of the Mediterranean populations, the olive oil is an integral part of Jordanian diets. Although figures may vary by time and place, olive oil contributes almost 20% of the total energy intake in Mediterranean menus (Kipnis *et al*., 1993). Thus, the effects of olive oil on health and disease have frequently been assessed through epidemiological studies focusing on the Mediterranean diet (Trichopoulou and Dilis, 2007). It has been proposed that the Mediterranean diet may be closer to the ancestral foods that were part of human development and our metabolism may have evolved to work optimally on such a diet rather than with the current diets richer in saturated fat and highly refined and processed foods. Therefore, it is possible that alleles that are associated with increase disease risk may be silenced in the presence of that more ancestral and traditional diet and lifestyle. This knowledge may provide the basis for successful public health as well individual approaches for disease prevention (Ordovas *et al*., 2007). Traditionally, olive oil has been the only alimentary fat containing primarily monounsaturated fat in its composition. Nowadays, other edible oils contain fatty acid compositions similar to that of the olive oil, namely high-oleic sunflower oil and rapeseed oil. However, olive oil has the exclusivity of being a real juice and its composition includes not only fat but also other multiple minor components with biological properties that are not present in any other edible oil. Chemically, olive oil is composed of triacylglycerols which account for about 98% of its total weight. In addition, it contains about 2% of other, nearly 250 minor components including aliphatic and triterpenic alcohols, sterols, hydrocarbons, volatile compounds and antioxidants (Servili and Montedoro, 2002; Servili *et al*., 2004; Tripoli *et al*., 2005; Covas *et al*., 2006). In particular, among the natural antioxidants of olive oil are carotenoids and phenolic compounds, which have both lipophilic and hydrophilic properties. Tocopherols are known as lipophilics, while phenolic alcohols and acids, hydroxy-isochromans, flavonoids, secoiridoids and lignans constitute the hydrophilic compounds. Natural antioxidants are reported to play a key role in preventing oxidation and have been already correlated to the storage stability of olive oils (Bendini *et al*., 2007; Baccouri *et al*., 2008; Nevado *et al*., 2009). The content of the minor components of an olive oil is strongly affected by many agronomical and technological factors, such as olive cultivar (Tura *et al*., 2007), the place of

cultivation (Vinha *et al*., 2005; Al-Maaitah *et al*., 2009), the climate, degree of maturation (Kalua *et al*., 2005; Dabbou *et al*., 2009), crop season (Gomez-Alonso *et al*., 2002; Ocakoglu *et al*., 2009), irrigation (Tovar *et al*., 2001) and the processing system (Ranalli *et al*., 2001; Ocakoglu *et al*., 2009) employed to produce the types of olive oil currently present on the market: extra-virgin, virgin, refined, ordinary, or pomace (Gimeno *et al*., 2002). Virgin Olive Oil (VOO) is that obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alteration in the oil. It has not undergone any treatment other than washing, decantation, centrifugation or filtration. Oils obtained using solvents, adjuvant, having a chemical or biochemical action, re-esterification process, or any mixture with oils of other kinds are excluded from this category (EEC, 2001). Extra-VOO is VOO with a free acidity, expressed as g of oleic acid/100 g of olive oil, less than 0.8 g. Virgin olive oil with an acidity greater than or equal to 3.3 is submitted to a refining process in which some components, mainly phenolic compounds and to a lesser degree squalene, are lost (Owen *et al*., 2000). By mixing virgin and Refined Olive Oil (ROO) an ordinary olive oil is produced and marketed. After VOO production, the rest of the olive drupe and seed is processed and submitted to a refining process, resulting in Pomace Olive Oil (POO), to which a certain quantity of VOO is added before marketing (International Olive Council, http://ucce.ucdavis.edu/files/filelibrary /2161/34496.pdf). Virgin olive oil is unique among edible vegetable oils because it is consumed without any refining process that would eliminate its natural flavors inherent part of which is the bitter taste. Taste is a very important factor among sensory liking characteristics that affects consumer preference as well as olive oil and food pairing (Boskou *et al*., 2005; Cerretani *et al*., 2008). The oxidative stability, sensory quality and health properties of virgin olive oil stem from a prominent and well-balanced chemical composition (Bendini *et al*., 2007; Esti *et al*., 2009; Inarejos-Garcia *et al*., 2009).

Elevated concentrations of in vivo circulating oxidized low density lipoprotein (oxLDL show a positive relationship with the severity of acute coronary events (Holvoet *et al*., 2001). Circulating oxLDL plasma concentrations were predictors for Coronary Heart Disease (CHD) both in CHD patients and in the general population (Meisinger *et al*., 2005; Wu *et al*., 2006; Ruano *et al*., 2007). The type of fat ingested is a key factor concerning LDL oxidation because it can modulate the susceptibility of LDL to undergo oxidative modification. Poly Unsaturated Fatty Acids (PUFA) rich in double bonds, are more prone to form conjugated dienes than Mono Unsaturated Fatty Acids (MUFA). A linoleic acid account for 90% of the PUFA presents in LDL and is the major substrate for its oxidation (Esterbauer *et al*., 1992; Bos *et al*., 2007). In most studies, oleic acid-rich LDL have been shown to be less susceptible to oxidation than linoleic acid rich LDL (Mata *et al*., 1997; Baroni *et al*., 1999; Fito *et al*., 2007). The oxidative modification of LDL plays a key role in atherosclerosis and CHD development (Hennig and Toborek, 2001). It is currently thought that oxLDL is more damaging to the arterial wall than native LDL (Fito *et al*., 2007). One of the earliest steps in the generation of oxLDL is the lipid peroxidation of PUFA. Tissue membranes that are rich in PUFA are more susceptible to oxidation by free radicals than membranes rich in MUFA (Reaven *et al*., 1994; Shimokawa, 1999) However, lipid peroxidation and its chain reaction in LDL, can be interrupted if LDL lipids are protected from free radicals by antioxidants. Olive oil is rich in MUFA and antioxidant compounds. The concentration of these antioxidants is influenced by the olive oil extraction procedures. Olive oil obtained exclusively by physical procedures, is much more than a MUFA fat because it contains relatively high amounts of antioxidants, mainly phenolic compounds have been shown to protect LDL from lipid peroxidation in vitro experiments (Owen *et al*., 2000; Visioli *et al*., 2000). It has displayed anti-thrombotic effects in cell culture and in vitro studies (Perez-Jimenez *et al*., 1999; Guzik *et al*., 2002; Duvall, 2005; Fito *et al*., 2005; Pacheco *et al*., 2006; Ruano *et al*., 2007; Kasdallah-Grissa *et al*., 2008). Compared with a saturated fat diet, olive oil- rich diet has been found to be associated with lower levels of blood pressure (Ruiz-Gutierrez *et al*., 1996; Ferrara *et al*., 2000). Hypertension is related to endothelial dysfunction which contributes to make the atherosclerotic plaque more unstable, thus increasing the risk of secondary events in CHD patients (Escobar, 2002; Perona *et al*., 2006).

Thus, the aim of the present study was to evaluate the effect of olive oils on lipid profile, oxidative stress and blood pressure in human.

MATERIALS AND METHODS

Subjects: Twenty five Jordanian (12 men and 13 women) from Alkarak city (Mutah and Almerj) participated in the study. Started from October/2008 to March/2009. The average age of men was 45 years (ranged from 37-50 years) with body mass index of 25.2 kg/m^2 (range, 23.4-27.2 kg/m^2) and of women was 39 years (ranged from 33-44 years), with body mass index of 26.3 kg/m^2 (range, 24.6-28.31 kg/m^2). All men and their wives were healthy. None of them showed evidence of chronic diseases (hepatic, renal, thyroid, cardiac), or family history of early onset cardiovascular disease. Venous blood was obtained from the fasting after an overnight period, at the beginning (base line) and end of each period of the study. Blood was collected in heparinized tubes and stored in containers with ice and kept in the dark. Special care was taken to avoid exposure to air, light and ambient temperature. Plasma was separated from whole blood by low-speed centrifugation at 1500 rpm for 20 min at 4°C and frozen at -20°C until analyzed.

Diet and design: Before participating in the study, all subjects were receive instruction on the basic concepts of food composition and characteristics, appropriate portions and recommended cooking techniques. Participants were given strict instructions about what to eat at home and were asked to replace their usual fat intake (butter, ghee, margarine, visible fat on meat and all oils) with the study olive oils. The participants were successively consumed three local, *balady*, virgin olive oils: High Phenolic Component Olive Oil (LPCO), Medium Phenolic Component Olive Oil (MPCO) and Low Phenolic Component Olive Oil (HPCO), purchased from local market in Alkarak city, for four weeks each. A 4-week washout period (basal lines) was included between the three experimental periods, consisting on the habitual dietary fat, comprised of hydrogenated, refined oil and a blend of seed oils. Body weight and height were recorded at the beginning and end of olive oil treatment until completion of the study and BMI were calculated. Before the beginning of the experimental study, the habitual dietary intake of the volunteers was recorded during four consecutive weeks (base line), using three- day- diet recall weekly and food frequency questionnaires. The same procedure was used during washout and experimental periods. This work showed that, there were no worthy differences in habitual diets of participants from week to week. During the experimental period, the only difference between the diets of participants was in the composition of the edible fats, added in the form of olive oils (LPCO, MPCO and HPCO) for cooking, salad dressing and occasionally for spreading on bread slices. The fatty acid composition of the three oils used in the study, as well as the minor component is shown in Table 1.

Intakes of protein, total carbohydrates, total fiber, fat, type of fat and micronutrients were assessed using food composition tables (Pellett and Shadarvian, 1970; Poul and Southgate, 1978). Energy content of daily food was calculated by multiplying the daily eaten carbohydrates, proteins and fats (grams) by 4, 4 and 9 Kcal, respectively Free acidity (% of oleic acid), Peroxide Value (PV) expressed (mEq O_2 kg^{-1} oil), K_{232} and K_{270} extinction coefficients calculated from absorption at 232 and 270 nm and β-sitosterol content were measured, following the analytical methods described in European Regulation, EEC (1991). Fatty acids methyl esters were formed according to the method of Lepage and Roy (1986). The fatty acid composition profiles of the oils were determined by gas chromatography according to the method described in EEC (1991). The concentrations of β-carotene and α-tocopherol in oils and blood lipid extracts were measured by High-performance Liquid Chromatography (HPLC) according to the method of Thurnham *et al.* (1988). Squalene content of the oils and plasma were determined by Gas-Liquid Chromatography (GLC) according to the methods of Miettinen (1988). Total phenols in oils and blood lipid extracts were measured spectnophotometrically according to the method of Singleton and Ross (1965) with phosphomolybdic phosphotungstic acid reagents. Plasma lipid peroxidation was determined by measuring by using the Thiobarbitunic Acid Reactive Substances (TBARS) assay, which measures Malondialdehyde (MDA) equivalents (Buege and Aust, 1978). This technique involves the spectrophotometric measurement of substances that react with thiobarbituric acid. Plasma Total Lipid (TL) concentration was measured according to the method of Sperry and Brand (1995). Total Cholesterol (TC) and Triglycerides (TG) concentrations in plasma were determined using the enzymatic methods from Arab Company for medical diagnostic, Jordan and Bicon, Germany, respectively. Concentrations of plasma Free Cholesterol (FC) and Phospholipids (PL) were measured according to methods mentioned by Tietz (1987). Plasma Cholesterol Ester (CE) concentration was calculated from the difference between TC and CE. Concentration of HDL-c in the plasma was measured by the precipitation technique using magnesium chloride and phosphotungstic acid from Bicon, Germany. Concentration of plasma LDL-c was calculated as the difference between TC and HDL-c using the formula of Friedewald *et al.* (1972), LDL-c = (TC) - (HDL-c) - (TG/5). Blood pressure measurements of participants were performed in the morning, after an overnight fast, at the right brachial artery in seated participants using a mercury-gauge sphygmomanometer. The measurements were recorded by the same nurse at the residential home at the beginning, middle and end of washout and both experimental periods. At each visit two blood pressure measurements were recorded and the average used to determine eligibility.

The data were expressed as Mean±Standard Deviation (SD). Statistical differences were determined by Duncan's multiple range tests at $p \leq 0.05$ by SAS Version (1988).

RESULTS AND DISCUSSION

Table 1 shows the physicochemical quality parameters and the composition of used olive oils. All the analyzed oils were met the legal limit values for VOO required by Regulation European Union (EEC, 2003) (acidity = 0.8%; peroxide value = 20 mequiv. O2 kg^{-1}; K_{270} = 0.22 and K_{232} = 2.5), indicating a low initial oxidation status, as was desired. These results show that the cultivar and factors affecting these analytical parameters had no significant influences (Kiritsakis *et al.*, 1998; Gomez-Alonso *et al.*, 2007). Given the object of this study, olive oil samples were selected on the basis of differences in natural antioxidant contents. The amounts of total phenols were significantly differed among analyzed oils. It ranged from 132 mg kg^{-1} in LPCO to 753 mg kg^{-1} in HPCO. As

Table 1: Quality indices, minor components (mg kg^{-1}) and fatty acid composition (%) of Low, Medium and High Phenols Containing Olive Oils (LPCO, MPC and HPCO) used as lipid sources in the study

Parameters	LPCO	MPCO	HPCO
Quality indices			
F A (% oleic acid)	0.41	0.38	0.37
P V (mEq O_2 kg^{-1})	11.6	10.8	10.2
K_{232}	2.03	1.92.	1.83
K_{270}	0.20	0.18	0.17
Minor components			
α-Tocopherol	264[b]	221[c]	319[a]
β-Carotene	3.72[b]	4.28[a]	3.31[c]
β-Sitosterol	1721	1847	1698
Squalene	3560	3730	3632
Total phenols	132[c]	368[b]	753[a]
Fatty acids			
C14:0	0.3	0.2	0.3
C16:0	10.9[ab]	10.6[b]	11.9[a]
C16:1	0.8	0.7	1.0
C18:0	5.4	5.0	5.2
C18:1(n9), O	71.8	72. 5	70.2
C18:2(n6), L	10.2	10.8	10.7
C18:3(n3)	0.50	0.40	0.30
C20:0	0.3	0.2	0.2
SFA	16.7[ab]	15.6[b]	17.8[a]
MUFA	72.6	73.2	71.2
PUFA	10.7	11.2	11.0
MUFA/SFA	4.30[ab]	4.65[a]	3.94[b]
MUFA/PUFA	6.79	6.54	6.47
O/L	7.04	6.71	6.65

PV: Peroxide Value. FA: Free Acidity. K_{232}, K_{270}: Specific extinction at 232 and 270 nm, respectively. SFA, MUFA and PUFA: Saturated, monounsaturated and polyunsaturated fatty acids, respectively. Significant differences in the same row are shown by different letters (a-c)

reported by different authors, the amount of total phenols is ranging between 50 and 1000 mg kg^{-1}, depending on various factors such as cultivar, climate, location, degree of maturation, type of crushing machine and oil extraction procedures. (Aguilera *et al*., 2005; Aparicio and Luna, 2002; Allalout *et al*., 2009). Phenolic compounds have a strong antioxidant and a free radical scavenging ability (Visioli *et al*., 1998). Moreover, their presence in olive oil contributes to the sensory characteristics, like its bitter, astringent and pungent taste (Gutierrez-Rosales *et al*., 2003). As previous investigations showed, the main determinants of virgin olive oil antioxidant activity are phenolic compounds that share o-diphenolic structures such as hydroxytyrosol and its derivates (Lavelli, 2002). The tocopherol fraction in virgin olive oils consisted mainly of α-tocopherols; these substances exert both vitamin potency and antioxidant action. There were significant differences in α-tocopherol amount between studied oils. In fact, α-tocopherol amounts are ranging from 221 mg kg^{-1} in MPCO to 319 mg kg^{-1} in HPCO. These results are in agreement with observations of Deiana *et al*. (2002) that α-tocopherol content is highly variety-dependent. Several authors reported a high correlation between phenol and α-tocopherol compounds and oxidative stability in virgin olive oil (Gomez-Alonso *et al*., 2002; Gomez-Alonso *et al*., 2007; Allalout *et al*., 2009). Table 1 also shows the pigment contents of studied oils. β-carotene concentration was 3.31 mg kg^{-1} in HPCO, 3.72 in LPCO mg kg^{-1} in and 4.28 mg kg^{-1} in MPCO. These results show that significant differences between the three oils were observed in pigment contents probably because the olives were at different stages of ripeness (Gomez-Alonso *et al*., 2007). These results are in agreement with the findings of Salvador *et al*. (2001), Psomiadou and Tsimidou (2001) and Allalout *et al*. (2009) which reported that the presence of the pigment in the oil depends on several factors, such as the olive cultivar, soil and climatic conditions, fruit ripeness and the processing procedures.

As shown in Table 1, the fatty acid composition, with a high percentage of oleic acid and low linoleic and linolenic acid contents, was in compliance with established limits (EEC, 2003) and with ranges depending to the varieties (Aranda *et al*., 2004). Oleic acid, the major Monounsaturated Fatty Acid (MUFA), showed limit variability among the three oil samples (about 72%). Relatively low percentages of Linoleic acid (about 11%), the major Polyunsaturated Fatty Acid (PUFA), were observed in the analyzed samples. Myristic, palmitoleic, stearic, linolenic and arachidic, showed no significant differences from one olive oil to the other. Levels of these fatty acids in the three tested oils were close to the IOC (2009). Variations in fatty acid composition observed in olive oil samples are probably related to both genetic factors and environmental conditions during the development and maturity of the fruit. These results are in agreement with the findings of other authors (Salvador *et al*., 2001; Morello *et al*., 2004; Allalout *et al*., 2009). They mentioned that several agronomic parameters could modify the fatty acid composition of olive oil. The percentages of Saturated Fatty Acids (SFA), MUFA, PUFA and the oleic acid/linoleic acid ratio (O/L) in the studied olive oils were also evaluated. It was observed that HPCO was rich in total SFA (17.8%) essentially due to its higher content in palmitic acid which represents the major acid of the SFA fraction. Concerning the total MUFA, PUFA and MUFA/PUFA ratio, there were no significant differences from one olive oil to the other. However, the MUFA/SFA ratio was significantly higher in MPCO (4.65) than in HPCO. The O/L ratio was 7.04 in LPCO, 6.71 in MPCO and 6.65 in HPCO. This ratio can be useful to characterize olive cultivars and to have a marked relationship with stability. Allalout *et al*. (2009) reported a positive correlation (r^2 = 0.7, $p \leq 0.001$) between this ratio and the oxidative stability of olive oil. Other authors showed that the O/L ratio was described as the main responsible factor for virgin olive oil oxidative stability (Aguilera *et al*., 2005).

Table 2: Calculated daily total energy, E (kcal) and macronutrients (% of E) intakes of men and women consumed Low, Medium and High Phenols Containing-olive Oils (LPCO, MPCO and HPCO) for 4-week*

Nutrients	Washout period 1	LPCO	Washout period 2	MPCO	Washout period 3	HPCO
Total energy						
Men	2460±35	2520±48	2510±33	2470±41	2490±38	2430±27
Women	2220±32	2270±40	2260±39	2240±30	2230±42	2280±35
Protein						
Men	10.2±0.4	10.5±0.5	10.1±0.8	10.7±0.3	10.3±0.7	10.5±0.3
Women	10.0±0.6	10.2±0.4	9.8±0.5	10.1±0.6	9.7±0.4	10.3±0.6
Carbohydrates						
Men	58.4±4.7	58.9±5.2	57.8±3.7	59.0±4.3	59.2±4.5	58.5±6.1
Women	57.7±5.3	56.6±4.3	57.4±4.5	57.4±5.1	57.3±4.8	57.5±4.7
Total fat						
Men	31.4±1.2	30.6±1.5	32.1±1.8	30.3±1.7	30.5±2.1	31.0±2.3
Women	32.3±1.5	33.2±1.7	32.8±2.4	32.5±1.6	33.0±1.8	32.2±2.0
SF						
Men	9.1±0.5	4.1±0.4**	9.5±0.7	5.1±0.3**	8.3±0.5	4.4±0.3**
Women	10.1±0.6	6.5±0.3**	10.6±0.4	5.5±0.2**	9.9±0.7	4.5±0.4**
MF						
Men	7.6±0.2	20.5±0.8**	7.1±0.4	19.8±0.7**	8.2±0.5	20.7±1.2**
Women	6.8±0.4	20.9±0.9**	7.4±0.2	20.3±1.4**	7.7±0.3	21.2±0.8**
PF						
Men	14.7±0.6	6.0±0.3**	15.5±0.8	5.4±0.4**	14.0±0.9	5.9±0.2**
Women	15.4±0.8	5.8±0.5**	14.8±1.3	6.7±0.3**	15.4±1.2	6.5±0.4**

*Data shown as means± SD. **: Differed significantly from the base line. SF, MF and PF: Saturated, monounsaturated and polyunsaturated fat. Washout Periods: 4-week of habitual diet pre olive oil administration

Table 2 shows the calculated daily energy intake and contribution of macronutrient in total energy of participants during basal and olive oil periods. As shown in this table, there were no significant differences in the calculated of daily energy intake and contribution of protein, carbohydrates and total fat in total energy between periods of olive oils and washouts. However, consumption of the three olive oils resulted in significant increase in monounsaturated fat and decrease in saturated and polyunsaturated fats contribution in total energy compared with washout periods. In general, the men received more energy than women (2480, 2250 kcal/day) and no markedly differences between them and women in contribution of macronutrients in total energy intake. A dietary survey showed that olive oils were consumed in a daily dose of about 70 g. It used at least twice a day, consistently for breakfast and lunch especially with chickpea and thyme and in this study used in cooking. All participants were complied with dietary and lifestyle recommendations and conserve their body weight during the study periods.

Table 3 shows the micronutrient intakes of participants during basal and olive oil periods. This table showed that there were no significant differences in intakes of fiber, cholesterol, β-carotene, vitamin A, α-tocopherol vitamin C and sodium between periods of olive oils and base lines. Consumption of studied olive oils resulted in significant higher intakes of squalene, β-sitosterol and total phenols than there base lines. However, no worth differences between men and women in intakes of micronutrients.

Table 4 shows the effects of intake of three olive oils on plasma lipid profile of men and women. Seven lipid classes were identified and quantified in the plasma of participants, for instance TG, PL, TC, FE, CE, LDL-c and HDL-c. There were no significant differences in TG, PL, TC, FC, or CE concentrations in plasma of participants consumed LMCO, MMCO or HMCO. However, statistically significant differences were observed in plasma LDL-c and HDL-c, HMCO consumption resulted in the highest reduction in LDL-c concentration (-12.2%) and rising in HDL-c concentration (+23.7%), whereas the LMCO consumption resulted in the lowest reduction and rising (-6.4 and +9). However, effects of MMCO consumption in these parameters were at the mid point. There was no worth noting differences in effects of these oils between men and women for that the given data were there mean. Finally, a statistically significant effect was observed upon TC/ HDL-c and LDL-c/HDL-c ratios, these being lowest in the HMCO treatment (3.62 and 2.10 for men; 3.25 and 1.77 for women). Recent works have shown increasing total and LDL-c concentrations after consuming olive oil, rich in oleic acid, compared to dietary oils rich in n-6 fatty acids (Howell *et al*., 1998; Pedersen *et al*., 2000) and others have reported greater cholesterol reductions of this oil (Sirtori *et al*., 1992; Madigan *et al*., 2000). These discrepancies may be due to differences in experimental conditions, including the employment of different varieties of olive oil (Perona *et al*., 2003; Perona *et al*., 2004). Present study showed that the olive oil intake had lowering effect on LDL-c and rising effect on HDL-c concentrations in the plasma of participants and the same observations were reported by Ruiz- Gutierrez *et al*. (1996) and Perona *et al*. (2004).

Table 5 shows the effects of intake of three olive oils on minor components in plasma of men and women.

Table 3: Estimated daily total fiber (g) and micronutrients (mg) intakes of men and women consumed Low, Medium and High Phenols Containing-Olive Oils (LPCO, MPCO and HPCO) for 4-week*

Nutrients	Washout period 1	LPCO	Washout period 2	MPCO	Washout period 3	HPCO
Total fiber						
Men	25.3±3.8	24.1±2.5	23.8±3.1	23.6±4.2	24.2±3.3	25.1±2.7
Women	23.7±4.2	25.0±2.8	24.6±4.0	25.2±3.3	23.4±2.4	24.5±3.5
Cholesterol						
Men	282±15	271±12	291±13	289±18	276±21	267±20
Women	296±18	279±22	305±16	295±15	290±17	278±13
β-Carotene						
Men	3.2±0.1	3.4±0.3	3.3±0.2	3.5±0.2	3.5±0.1	3.7±0.3
Women	3.5±0.4	3.5±0.3	3.4±0.3	3.5±0.2	3.6±0.4	3.6±0.3
Vitamin A (RE)						
Men	410±15	430±13	385±17	428±15	393±11	416±10
Women	392±16	411±14	367±12	403±11	378±16	421±15
α-Tocopherol						
Men	14.2±0.8	14.4±0.7	13.8±1.2	14.1±1.0	14.6±0.9	14.8±1.1
Women	14.4±1.1	14.5±1.0	14.2±1.4	14.6±1.3	14.8±1.2	14.6±0.7
Vitamin C						
Men	55.8±4.3	57.3±3.7	61.4±3.2	58.2±4.5	54.7±3.9	55.1±3.2
Women	56.3±3.5	59.5±4.1	60.2±3.7	59.6±3.3	57.8±2.8	60.2±4.4
Squalene						
Men	160±11	270±14**	175±9	267±15**	158±11	282±12**
Women	143±9	258±15**	131±8	251±13**	154±9	265±11**
β-Sitosterol						
Men	109±5	123±6**	112±7	115±5	105±8	133±10**
Women	102±7	116±5**	107±8	111±7	104±6	123±11**
Phenols						
Men	7±1.2	16±2.5**	6±1.3	34±3.7**	8±2.0	56±4.5**
Women	6±0.9	14±2.6**	8±1.5	27±2.9**	8±2.1	53±5.1**
Sodium						
Men	803±23	764±31	781±34	831±22	745±19	783±27
Women	758±25	803±40	823±18	775±36	767±32	834±30

*Data shown as means± SD. **: Differed significantly from the base line. Washout periods: 4-week of habitual diet pre olive oil administration

Present study aimed at analyzing the effect of the non glyceride fraction of olive oil as a whole. Nevertheless, the three different experimental olive oils employed had different concentrations of β-carotene, α-tocopherol, β-sitosterol, squalene and total phenols. The amount of squalene, the main hydrocarbon in VOO, has been considered hypercholesterolemic compared with other oils (Pedersen *et al*., 2000; Perona *et al*., 2004), in plasma of the subjects was increased after consumption of olive oil especially after HPCO. However, there were no markedly differences in squalene content in the studied oils, it ranged from 3560 mg kg^{-1} in LPCO to 3730 mg kg^{-1} in MPCOO (Table 1) and in estimated squalene intake, it ranged from 259 mg/day for MPCO to 273 mg/day for HPCO (as average for men and women) (Table 3). The effect of oil type on plasma squalene level was took the order: HPCO> LPCO> MPCO (Table 5). Thus, it appears that this constituent had a minor role, if any at all, on the changes in the plasma lipid profile observed (Table 4). The same observation was mentioned by (Mangas-Cruz *et al*., 2001). Nakamura *et al*. (1997), they reported that the oral administration of squalene at different dose has no effect on serum lipid in rats. Table 5 also shows that the plasma levels of α-tocopherol retinol and β-carotene were nonsignificantly increased after consumption of olive oils compared with the beginning line. Back to the Table 1, this table shows that the amount of α-tocopherol in the three experimental oils was took the order: HMCO> LMCO> MMCO and of β-carotene was took the order: MMCO> LMCO> HMCO whereas the effect of these oils on blood lipids was followed the order: HMCO> MMCO> LMCO (Table 4). Moreover the concentrations of both vitamins in the plasma were not significantly affected by either of these oils which suggest that these constituents were not responsible for the effect on plasma lipid concentrations. It is known that the main effect of β-sitosterol is preventing the absorption of dietary cholesterol from the gut. It has a hypocholesterolemic effect on plasma TC and LDL-c (Heinemann *et al*., 1991; Mangas-Cruz *et al*., 2001). As the three olive oils employed were devoid of cholesterol, no such effect was possible in the current study. Thus, among the minor component in olive oil, the phenols could be responsible for the observed effect upon plasma lipid. Data showed that the amount of total phenol compounds in three studied olive oils (Table 1) and in subjects plasma (Table 5) was significantly followed the order: HMCO> MMCO> LMCO and the effects of these oils in LDL-c reduction and HDL-c rising was followed the same order

Table 4: Plasma lipid profile (mg dl^{-1}) of men and women consumed Low, Medium and High Phenols Containing- Olive Oils (LPCO, MPCO and HPCO) for 4 week*

Parameters	Base line	After LPCO (L)	Base line	After MPCO (M)	Base line	After HPCO (H)	Changes (%) after		
							L	M	H
TG									
Men	120±7	119±8	118±6	116±5	124±8	121±5	-0.8	-1.7	-2.4
Women	127±6	124±7	121±5	117±8	123±7	123±6	-2.4	-3.2	-
PL									
Men	109±5	108±7	111±6	109±4	110±5	110±5	-0.9	-1.8	-
Women	110±7	107±6	109±4	110±5	108±5	110±4	-2.7	+0.9	+1.8
TC									
Men	174±7	170±6	172±7	167±9	172±6	166±9	-2.3	-2.9	-3.5
Women	169±8	165±9	170±8	166±7	169±9	167±8	-2.4	-2.4	-1.2
FC									
Men	51±3	49±3	50±3	47±5	51±3	47±4	-3.9	-6.0	-7.8
Women	50±4	47±3	52±3	49±4	52±4	50±3	-6.0	-5.8	-3.8
CE									
Men	122±6	121±6	122±5	120±7	121±6	119±8	-0.8	-1.6	-2.5
Women	119±7	118±5	118±7	117±7	117±8	117±6	-0.8	-0.8	-
LDL-c (L)									
Men	114 ±5	107±4**	111±4	101±5**	110±5	96±6**	-6.1^{a}	-9.0^{b}	-12.7^{c}
Women	104±5	97±4**	105±5	96±6**	103±4	91±8**	-6.7^{a}	-8.6^{ab}	-11.7^{c}
HDL-c(H)									
Men	36.0±3	39.2±3**	37.4±4	42.8±3**	37.2±5	45.8±4**	$+8.9^{c}$	$+14.4^{b}$	$+23.1^{a}$
Women	39.6±4	43.2±4**	40.8±3	46.6±3**	41.4±4	51.4±5**	$+9.1^{c}$	$+14.2^{b}$	$+24.2^{a}$
TC/H									
Men	4.83±0.2	4.33±0.3**	4.60±0.4	3.90±0.2**	4.62±0.3	3.62±0.2**	-10.4^{a}	-15.2^{b}	-21.6^{c}
Women	4.27±0.3	3.82±0.2**	4.17±0.2	3.56±0.3**	4.08±0.3	3.25±0.4**	-10.5^{a}	-14.6^{b}	-20.3^{c}
L/H									
Men	3.17±0.1	2.73±0.2**	2.97±0.3	2.36±0.1**	2.96±0.3	2.10±0.2**	-13.8^{a}	-20.5^{b}	-29.1^{c}
Women	2.63±0.1	2.25±0.1**	2.57±0.2	2.06±0.2**	2.49±0.1	1.77±0.3**	-11.0^{a}	-19.8^{b}	-28.9^{c}

*Data shown as means± SD. **: Differed significantly from the base line. Significant differences in effects of the three oils are shown by different letters (a-c). TG, PL, TC, FC, CE, LDL-c and HDL-c: Triglycerides, Phospholipids, Total Cholesterol, Free Cholesterol, Cholesterol Ester, Low Density Lipoprotein- Cholesterol and High Density Lipoprotein-cholesterol, respectively

(Table 4). The same observation was mentioned by (Mangas-Cruz *et al*., 2001).

Table 5 shows the effects of intake of three olive oils on oxidation status of participants' plasma. Lipid oxidation, a process mediated by free radicals, is considered to be important in the development of atherosclerosis. Lipid may be protected against attacks of free radicals by antioxidants in plasma. There are a growing number of studies indicating that antioxidants may be responsible for some of the protective effects of olive oil (Giugliano, 2000; Moline *et al*., 2000). One way of estimating free radical activity is to determine the concentration of MDA in plasma which is byproduct of lipid peroxidation. In this study, olive oil intake resulted in lower plasma MDA concentrations compared with the beginning lines. However, there were significant differences in effects of the three studied olive oils in this oxidation product, there reducing effect followed the order: HMCO> MMCO>LMCO, which parallel there effects in blood lipids. The same observation was obtained from studies in animals (Del Boccio *et al*., 1990; Yu *et al*., 1993) and humans (Yalcin *et al*., 1989; Hargrove *et al*., 2001) have shown that there is a close relationship between lipid peroxidation and hypercholesterolemia. Halliwell and Chirico (1993), demonstrated higher stability of saturated and monosaturated oils in lipid peroxidation as compared to polyunsaturated oils. Lipoproteins rich in MUFA after long term consumption of olive oil have been shown to be less susceptible to oxidation (Hargrove *et al*., 2001). Ohrvall *et al*. (1994) states that MDA concentrations in plasma are inversely correlated to the proportion of PUFA in blood lipoprotein lipids. His findings suggested that other factors, such as, the availability of antioxidants, polyphenols and others, may be of greater importance for intravascular lipid peroxidation. Olive oil has the biophenols (Moline *et al*., 2000; Mangas-Cruz *et al*., 2001), β-carotene and tocopherols (Rosengren *et al*., 1999; Chen *et al*., 2000) which were shown to improve in vivo an antioxidant defenses and protect LDL from oxidant phenomena, a condition necessary for the formation of athermanous plaque.

Table 5 shows the effects of intake of three olive oils upon blood pressure. It is probable that arterial hypertension resulted to be quantitatively the most important risk factor for CHD due to its repercussion on cardiovascular mortality (Aranda, 1996). However, there were increasing evidence showing that olive oil reduces systolic and diastolic blood pressure in normotensive and hypertensive individuals (Ruiz-Gutierrez *et al*., 1996;

Table 5: Plasma malondialdehyde, MDA (μmol L^{-1}l) and antioxidant compounds (μg ml^{-1}) and blood pressure, BP (mmHg) of men and women consumed Low, Medium and High Phenols Containing-Olive Oils (LPCO, MPCO and HPCO) for 4-week*

Parameters	Base line	After LPCO (L)	Base line	After MPCO (M)	Base line	After HPCO (H)	Changes (%) after L	M	H
MDA									
Men	0.92±0.02	0.86±0.03**	0.89±0.02	0.78±0.01**	0.91±0.03	0.76±0.03**	-6.5^{a}	-12.4^{b}	-16.5^{c}
Women	0.93±0.03	0.87±0.01**	0.92±0.01	0.81±0.02**	0.90±0.04	0.74±0.03**	-6.5^{a}	-12.0^{b}	-17.7^{c}
α-Tocopherol									
Men	4.9±0.3	5.0±0.4	5.1±0.3	5.2±0.2	4.8±0.2	4.8±0.3	2.0	2.0	-
Women	4.6±0.3	4.7±0.2	5.0±0.2	5.0±0.4	5.1±0.3	5.0±0.4	2.2	-	-2.0
Retinol									
Men	2.4±0.3	2.5±0.2	2.5±0.1	2.6±0.2	2.7±0.2	2.7±0.1	4.2	4.0	-
Women	2.3±0.1	2.3±0.3	2.6±0.2	2.7±0.2	2.5±0.3	2.5±0.1	-	3.8	-
β-Carotene									
Men	0.27±0.03	0.28±0.01	0.28±0.01	0.29±0.02	0.30±0.02	0.32±0.01	3.7	3.6	6.7
Women	0.28±0.02	0.30±0.01	0.29±0.02	0.30±0.01	0.29±0.01	0.30±0.02	7.1	3.4	3.4
Squalene									
Men	0.32±0.02	0.36±0.04**	0.33±0.04	0.35±0.02	0.34±0.03	0.40±0.02**	12.5^{ab}	6.1^{b}	17.6^{a}
Women	0.34±0.04	0.36±0.02	0.35±0.03	0.37±0.04	0.34±0.02	0.39±0.03**	5.9^{b}	5.7^{b}	14.7^{a}
PC (mmolL^{-1})									
Men	0.28±0.03	0.31±0.03**	0.30±0.04	0.37±0.03**	0.31±0.03	0.41±0.04**	10.7^{c}	23.3^{b}	32.3^{a}
Women	0.27±0.02	0.31±0.04**	0.29±0.02	0.36±0.03**	0.30±0.01	0.41±0.04**	14.8^{c}	24.1^{b}	36.7^{a}
Systolic BP									
Men	136±4	132±7	135±5	126±4**	135±6	124±8**	-2.9^{a}	-6.7^{ab}	-8.1^{b}
Women	135±6	130±5	134±6	126±8**	135±5	125±6**	-3.7	-5.9	-7.4
Diastolic BP									
Men	84±4	80±3	83±6	76±4**	84±5	75±4**	-4.8^{a}	-8.4^{ab}	-10.7^{b}
Women	83±5	80±4	84±5	78±4**	84±4	74±6**	-3.6^{a}	-7.1^{ab}	-11.9^{b}

*Data shown as means±SD. **: Differed significantly from the base line. Significant differences in effects of the three oils are shown by different letters (a-c). PC: Phenolic Compounds

Ferrara *et al*., 2000). The present study was designed to assess the effects of three olive oils with different levels of minor compounds on plasma lipid composition and oxidation and blood pressure of healthy volunteers. Systolic and diastolic blood pressures were reduced in participants after consuming these oils and this reduction was increased with increasing phenols level in the oil. However, some studies failed to find effects of fish oil or n-3 fatty acid supplementation on blood pressure in treated subjects when compared to VOO as a placebo, suggesting that VOO is able to reduce blood pressure to a similar extent. Previously, Ruiz- Gutierrez *et al*. (1996) and Perona *et al*. (2004) had observed a hypotensive effect of dietary VOO compared to oleic acid-rich oil, such as High Oleic Acid Sunflower Oil (HOSO). Although it needs to be elucidated which of the minor constituents of VOO are responsible of these effect, on the basis of the accumulated data, the PC may play a key role in this regard, since these compounds are absent in HOSO. The mechanism of reduction of blood pressure by dietary olive oil has been related to endothelium-dependent vasorelaxation via enhanced nitric oxide (Ruiz-Gutierrez *et al*., 1996; Ferrara *et al*., 2000).

Conclusion: Diet rich in olive oil, containing high minor compounds, especially phenols, produce beneficial modification in plasma lipid profile and oxidation in healthy subjects. Dietary olive oil is helpful in reducing the systolic and diastolic pressures However, among the minor components, the phenolic compounds have the significative beneficial effect in these parameters which imply that the daily ingestion of food rich in these compounds is necessary to produce an accumulative desirable effect.

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