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RAPID COMMUNICATION

# Effects of extra virgin olive oil supplementation at two different low doses on lipid profile in mild hypercholesterolemic subjects: A randomised clinical trial

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**ABSTRACT. Olive oil, the principal fat of Mediterranean Diet, is known to improve several cardiovascular risk factors at relatively high doses togheter with intensive modifications of dietary habits. Since this is hard to obtain in the long term, an intervention with encapsulated oil supplements might be more feasible. Aim of this preliminary study was to investigate the effects of the supplementation of a moderate amount of encapsulated extra virgin olive oil *vs* a lower dose in mildly hypercholesterolemic subjects, as part of their estabilished diet, on blood lipid profile. A prospective randomized study was performed. Thirty-four mildly hypercholesterolemic subjects [age, mean±SD: 46±7yr; total cholesterol (TC): 235±28mg/dl] were randomly assigned to receive 2 g (group A) or 4 g (group B) per os of extra-virgin olive oil for 3 months. TC, triglycerides (TG), LDL cholesterol, HDL cholesterol, apolipoprotein A1 (Apo-AI), apolipoprotein B (Apo-B), and atherogenic index of plasma (AIP) were evaluated at the beginning and at the end of the study. In group B, but not in group A, a significant reduction of Apo-B values (7%) was observed; TG concentrations showed a trend towards reduction and Apo-A1 values a trend towards increase (9%). A significant decrease in Apo-B/Apo-A1 ratio ($p<0.01$) was also observed in group B. Extra virgin olive oil supplementation significantly decreased AIP from baseline in group B ($p<0.05$). The results of the present study seem to suggest that the daily supplementation, on top of the normal diet, of at least 4 g of extra virgin olive oil, in mildly hypercholesterolemic subjects, is associated to favorable modifications of the plasmatic lipid profile.**
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## *INTRODUCTION*

Coronary artery disease (CHD) represents an important cause of death in Italy as well as in United States and in Western countries (1, 2). Hypercholesterolemia, and recently apolipoprotein B (Apo-B)/apolipoprotein A1 (Apo-A1) ratio and levels of small dense LDL, are estabilished cardiovascular risk factors (3, 4).

Olive oil intake, on the other hand, has been reported to be protective on cardiovascular disease (CVD) (5) improving blood lipid profile (6) and favorably affecting platelet functions (7). Moreover, extra virgin olive oil rich in $\alpha$-tocopherol and phenolic compounds has shown to possess anti-oxidant (8) and anti-inflammatory (9) properties.

However, high doses of olive oil have been used in these studies, employing experimental diets with well-controlled compositions of foods and fats, limiting the feasibility of these interventions 'in real life'.

Thus, we sought to evaluate if a novel formulation of a relatively small dose of encapsulated extra virgin olive oil (2 g daily *vs* 4 g), as a supplementation to a regular diet, has similar beneficial effects on lipid profile of moderately hypercholesterolemic middle-aged subjects.

**Key-words:** Apolipoprotein B, cholesterol, olive oil.

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## *SUBJECTS AND METHODS*

Partecipants were selected from December 2004 to March 2005 among employees of the Santa Croce e Carle Hospital of Cuneo. Inclusion criteria were: 30 to 65 yr of age, a total cholesterol (TC) level ≥200 mg/dl; exclusion criteria were: history of CVD, any severe chronic illness (including diabetes mellitus and hypertension), use of drugs affecting lipid metabolism. A sample size of 15 patients for each group of oil supplementation was required to detect a reduction of 10% TC levels at the end of the treatment (from baseline), at 0.05 significance level, with 90% power. Sample sizes were set at 17 patients per group, allowing for possible dropouts. The institutional review board approved the study; the first consecutive 34 hypercholesterolemic eligible employees provided informed consent to partecipate to the study and were enrolled.

Before initiation of the study, each subject was examined by a physician of the health-care service of the hospital and filled up a questionnaire about dietary habits, dressing comsumption, physical activity, consumption of voluptuary substances, existence of other diseases, and assumption of drugs.

Blood pressure was measured after 5-min seated rest using the sphygmomanometric cuff method. This measurement was repeated after 1 min, and the mean value was used. Height and weight were determined by the same physician according to a standard protocol in order to calculate the body mass index (BMI).

We advised the subjects to keep their level of physical activity and dietary habits constant during the study and to record every event that could influence the metabolic parameters, like variations of the consumption of alcohol or cigarettes or any important pathological or stressful event.

The food dressing most widely used by the subjects was olive oil. They utilized also, but in smaller measure, other vegetal oils or animal fats. None of them reported significant changes in the type and in the amount of dressing used throughout the study.

### Study design

A prospective randomized study was performed. The volunteers were randomly divided into 2 groups, each consisting of 17 subjects. Each subject assumed, for 3 consecutive months, per os, fasting, in the morning, 2 (group A) or 4 (group B) capsules, respectively, each containing 1 g of extra virgin olive oil. All the subjects have been supplied with bottles containing the amount of caps sufficient to complete the whole period of the study.
The olive oil used in the present study is a variety of extra virgin oil, naturally rich in phenolic compounds, provided by Alex Nember s.r.l. ("Fattoria Paradiso", Raffa di Puegnago, Brescia, Italy). The predominant components of the olive oil are: 187 mg/kg of poliphenols, 5 mEq O2/kg of peroxides, a concentration of mono-unsaturated fatty acids (MUFA) over 80% (oleic fatty acid =79.4%), of poly-unsatured fatty acids (PUFA) over 5% (linoleic acid =4.7% and linolenic acid =0.55%), and of satured fatty acids <14% (palmitic acid =11.9%). Oil composition was assessed by gas and liquid chromatography and mass spectrometry standard analysis. The capsules were gelatin-coated, transparent, and yellow.
The 34 subjects that completed the study did not show any side effect.
The compliance of the volunteers towards the assumption of the prescribed doses of oil has been estimated through the verification of the number of caps remained in the bottles to the term of the study and it has been considered satisfying.

### Measurements

Venous blood samples were collected into EDTA-containing tubes from all subjects after a 12-h overnight fast, at the start and at the end of the study. Plasma was obtained by low-speed centrifugation for 15 min at 4 C within 30 min of venipuncture. To reduce interassay variation, plasma was stored at –80 C and analyzed at the end of the study.
TC, triglycerides (TG), HDL, Apo-A1, and Apo-B were measured in the laboratory of the hospital, according to the Manual of Laboratory Operations: Lipid Research Clinics Program (10). In particular, plasma TC and TG levels were determined with standard enzymatic colorimetric methods, HDL with an enzymatic-colorimetric method after precipitation with $Mn^{2+}$, Apo-A1, and Apo-B with a turbidimetric method (BM/hitachi 717, BBR).
LDL cholesterol concentration was obtained using the Friedwald formula (11) = TC–HDL–(TG/5) (all partecipants had TG levels <400 mg/dl).
Atherogenic index of plasma (AIP) was computed according to the following equation (12):
AIP=log(TG/HDL) [with units for TG and HDL in mmol/l].
AIP has been demostrated to possess a close relationship with LDL particle size, being so useful in predicting plasma atherogenicity (12).

### Statistical analyses

Data were tested for normal distribution by the Shapiro-Wilk's test. Paired t tests, unpaired t tests, the Mann-Whitney U test (unpaired samples) or Wilcoxon's signed-rank test (paired samples) were used where appropriate. Normally distributed data are presented as means±SD. Data not normally distributed are expressed as median (25-75 percentiles).The $\chi^2$ test was used to determine frequency differences. A $p$ value <0.05 was considered statistically significant.
Statistical analysis was performed by using SPSS for WINDOWS (version 13.0; SPSS Inc. Chicago).

## *RESULTS*

The characteristics of the Study Population at baseline were the following (mean±SD): age = 46±7 yr, BMI=24.1±3.14 kg/m$^2$, SBP=124±15 mmHg; DBP=79±8 mmHg; TC=235±28 mg/dl; TG (median, 25-75 percentiles) =117, 65-339 mg/dl; HDL=67±13 mg/dl, LDL=142±30 mg/dl; Apo-A1=1.81±0.34 g/l, Apo-B=1.24±0.23 g/l.
Dietary and voluptuary habits, anthropometric data, blood pressure data did not showed significant differences between the group A and group B.
In group B, Apo-B levels significantly decreased ($p$<0.05), of about 7% from the baseline value; in the same group Apo-A1 levels increased, on average, of 8.9% ($p$=0.08), while mean TC and LDL cholesterol levels decreased only by 3% ($p$=ns) and TG levels by 11.5% ($p$=0.07). A significant decrease of Apo-B/Apo-A1 ratio ($p$=0.015) was also observed in group B (Table 1).
Four grams of extra virgin olive oil supplementation significantly improved AIP ($p$<0.04).
We did not find any significant difference in the lipid profile in the group A.

Table 1 - *Effect of 3-month treatment with 2 doses of extra virgin olive oil on lipid profile (mean±SD values or median*, 25-75 percentiles).*

| Variables | 2 cps | | | 4 cps | | |
|---|---|---|---|---|---|---|
| | Baseline | End | $p$* | Baseline | End | $p$** |
| Cholesterol (mg/dl) | 237±31 | 237±44 | ns | 234±25 | 227±21 | ns |
| Triglycerides (mg/dl)* | 111 (65-250) | 115 (68-241) | ns | 120 (66-339) | 111 (60-302) | 0.07 |
| HDL (mg/dl) | 68±15 | 66±15 | ns | 66±12 | 67±14 | ns |
| LDL (mg/dl) | 142±33 | 144±46 | ns | 143±27 | 138±26 | ns |
| Apo-A1 (g/l) | 1.84±0.40 | 1.88±0.32 | ns | 1.78±0.29 | 1.94±0.33 | 0.08 |
| Apo-B (g/l) | 1.23±0.23 | 1.19±0.27 | ns | 1.24±0.23 | 1.15±0.17 | 0.03 |
| AIP | 0.10±0.23 | 0.08±0.22 | ns | 0.15±0.23 | 0.10±0.25 | 0.039 |

**$p$: significance level *vs* baseline. Apo-A1: apolipoprotein A1; Apo-B: apolipoprotein B.

## DISCUSSION

In this study we have found a beneficial effect of dietary supplementation with at least 4 g of encapsulated extra virgin olive oil on blood lipids in mild hypercholesterolemic subjects. In particular, the higher dose of 4 g of encapsulated oil resulted in significant improvement of Apo-B levels, of Apo-B/Apo-A1 ratio and of AIP.

Hypercholesterolemia, and mainly high concentrations of LDL cholesterol, are generally accepted as a strong risk factor for atherosclerotic CVD (3). Apo-B is the major lipoprotein among all atherogenic lipoproteins (3). Several studies suggest that Apo-B levels could improve the prediction of risk of CHD (4). Apo-A1 is carried in anti-atherogenic particles such as HDL. Low levels of Apo-A1 are associated with increased risk for CHD (3). Recent studies have shown that Apo-B/Apo-A1 ratio represents a better marker of risk for CVD than lipids and lipoproteins (4).

Since standard and inexpensive methodologies for the measurement of the pattern B of LDL particles are not available, several surrogate markers of the so called small dense phenotype of LDL, have been proposed. Among them, Dobiasova and Frohlich (8) proposed the equation named AIP, defined as log(TG/HDL). These authors demostrated that AIP is inversely correlated with LDL particle size, being so useful in predicting plasma atherogenicity (8).

Our data accord with and extend previous studies in which the beneficial effect of olive oil, as part of the Mediterranean Diet, has been investigated (6-9). However, some of these studies investigated different types of subjects (i.e. healthy subjects or healthy men with a first-degree relative with previous myocardial infarction) and higher doses of olive oil (10-fold with respect to our doses) were used. Moreover, extensive modifications of dietary habits were advised in these trials. For all these reasons, the significantly favorable effects of olive oil previously reported could be likely difficult to be achieved in everyday life long term.

Our data for the first time show that this new formulation of an encapsulated extra virgin olive oil particularly rich in polifenol compounds at relatively low doses, maintains (in mildy hypercholesterolemic subjects) the aforementioned antiatherogenic effects on lipid profile achieved by higher doses of olive oil. These results have been obtained without intensive changes of dietary habits, suggesting that this type of intervention could be more feasible in everyday life especially for working adults.

These findings could be partly explained with the overall better biochemical properties of the extra virgin olive oil employed in the study in comparison to other similar oils, in particular regarding polyphenols, peroxides, MUFA, and PUFA content. These features could be due to the peculiar manual modalities of olive harvest, the technique of cold pressing and the organoleptic characteristics of these olives, that contain many anti-oxidant substances.

In our opinion, similar non-farmacological intervention strategies could be taken into account for primary prevention of CVD in selected subjects with low cardiovascular risk. In these subjects the low cardiovascular risk may not justify a pharmacological intervention, such as therapy with statins or other antilipemic drugs, that can determine also a high risk of adverse effects.

Ultimately, we should aknowledge some limitations of our study. We did not obtain neither a significant reduction of TC nor of LDL levels: nevertheless, we think that a significant change in Apo-B levels and Apo-B/Apo-A1 ratio could represent an improvement of the atherogenic profile. Moreover AIP modifications, which reflect an increase of LDL particle size, could represent a qualitative improvement (i.e. anti-atherogenic) of the lipid status.

In conclusion, our data suggest that in mildly hypercholesterolemic subjects, it is possible to obtain positive effects on the lipid profile, only adding to the normal diet at least 4 g of encapsulated extra virgin olive oil, without modifying the usual alimentary habits and without assuming drugs.

The demonstration of the effectiveness of a preventive intervention of atherogenic disease represents an incentive to study this topic in depth. Future research will be addressed to examine the metabolic effects of larger doses of extra virgin olive oil, in subjects belonging to groups with different anthropometric features at different risk of CVD.

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