

VOL. 135 SUPPL. 1

DECEMBER 1997

ISSN: 0021-9150
ATHSBL 135 (SUPPL. 1) S1-S20

*International Journal for Research
and Investigation on
Atherosclerosis and Related Diseases*

atherosclerosis

*Affiliated with the
International
Atherosclerosis Society*



**Supplement
XI National Congress of the Italian Society
for the Study of Atherosclerosis
Vibo, Valentia
1-2 December 1997**

e l s e v i e r

ANTIOXIDANT AND OTHER BIOLOGICAL PROPERTIES OF OLIVE OIL POLYPHENOLS

F. Visioli and C. Galli, Institute of Pharmacological Sciences, Via Balzaretti 9, 20133 Milano, Italy

Reactive oxygen species are implicated in the pathogenesis of cancer and coronary heart disease (CHD). Cardiovascular disease is lowest among people that follow the Mediterranean diet, that abounds in grain, fruit, vegetables, and olive oil. Extra-virgin olive oil is rich in phenolic antioxidants that are responsible for its stability and taste. The possible antioxidant activity of these compounds on uncontrolled oxidative processes in the cardiovascular system has been tested *in vitro*. Also, the effects of olive oil phenolics on the production of nitric oxide, which plays a role in host defense against parasites, were investigated. In order to assess the biological effects of olive oil phenols, we tested pure compounds (hydroxytyrosol - HT, oleuropein - OE) on a model of human LDL oxidation. Several markers of oxidative stress, concerning both lipids and proteins, were evaluated. HT and OE inhibited LDL oxidation at concentrations of 10^{-5} M. This protective effect was also noted when oxidation was triggered by the metal-independent system hydrogen peroxide-horseradish peroxidase, indicating both a chelation of free metal ions and a radical scavenging activity of olive oil phenolics. Furthermore, OE markedly amplified NO production in LPS-stimulated murine macrophages ($+60 \pm 5.2\%$; $n = 28$). This effect was dose-dependent and was reversed by the NO synthase inhibitor L-NAME, indicating that OE increases the activity of macrophage iNOS. Moreover, Western blot analysis of mac-iNOS demonstrated that oleuropein also enhances its expression. The results demonstrate that olive oil-derived phenols prevent LDL oxidation at low concentrations (10^{-5} M) and increase the production of a bactericidal and vasorelaxant factor, such as NO, also shown to inhibit LDL oxidation. Taken together, these data may partly explain the observed low incidence of CHD in the Mediterranean area.

ABSTRACTS

LIMITED CONFORMATIONAL FLEXIBILITY OF A DISULFIDE-LINKED APOLIPOPROTEIN A-I DIMER IN RECONSTITUTED HIGH DENSITY LIPOPROTEINS

S. Airoldi, L. Calabresi, *G. Vecchio, D. Galbiati, F. Frigerio, C. R. Sirtori and G. Franceschini. Center E. Grossi Paoletti, Institute of Pharmacological Sciences, University of Milano, and *Istituto di Chimica degli Ormoni, Consiglio Nazionale delle Ricerche, Milano.

Apolipoprotein A-I_{Milano} (apoA-I_M) is a molecular variant of apoA-I characterized by the Arg¹⁷³→Cys substitution. The disulfide-linked dimer of apoA-I (A-I_M/A-I_M) has been purified from carriers plasma and expressed in *E. coli*. In order to examine the effects of the introduction of an interchain disulfide bridge on the lipid binding properties of apoA-I, we evaluated the structure and properties of reconstituted HDL (rHDL) containing palmitoyl-oleoyl-phosphatidylcholine (POPC) and either apoA-I or A-I_M/A-I_M. We observed that, differently from apoA-I, A-I_M/A-I_M forms only two species of rHDL particles despite a wide range of initial POPC to protein ratios: one have a diameter of 7.8 nm and contains 1 molecule of A-I_M/A-I_M, and one have a diameter of 12.5 nm and contains 2 molecules of the dimer. The particles were compared with two apoA-I rHDL particles of similar sizes using spectroscopic techniques and limited proteolysis. The analysis of the A-I_M/A-I_M structure revealed that the protein adopts the same conformation in both large and small rHDL, with 12 helices, 6 per each monomer, associated with the lipid bilayer. ApoA-I, instead, assumes two different conformations, with 6 and 8 helices interacting with lipids, in the small and large particle respectively. These findings suggest that the dimerization of apoA-I, by the introduction of an interchain disulfide bridge does not affect the ability of the protein to form discoidal rHDL, but remarkably reduces the conformational flexibility of the lipid-bound molecule, thus probably affecting its functionality in lipid metabolism.

CAROTID ARTERY IMT MEASURED WITH AN ELECTRONIC CALIPER IN PATIENTS ATTENDING A LIPID CLINIC

D. Baldassarre, M. Amato, E. Tremoli and C.R. Sirtori. Center E. Grossi Paoletti, Institute of Pharmacological Sciences, University of Milan, Via Balzaretti 9, Milan, Italy.

Intima-media thickness (IMT) of the carotid artery is considered a marker of carotid/coronary atherosclerosis and it is widely used in pharmacological and clinical studies to follow the natural, pathological or pharmacological induced changes of arterial walls. previous studies have been performed by measuring IMT with highly sophisticated techniques usually not available in the normal clinical practice where, instead, a simple electronic caliper is generally used. Aim of the present observational study was to evaluate whether data obtained by measuring the IMT with an electronic caliper can be considered as representative of those obtained by using more sophisticated methods for IMT measurement. Ultrasonographical and clinical data relative to 963 patients attending the Lipid Clinic of the Enrica Grossi Paoletti Center in Milan have been analysed. Specifically, it has been evaluated whether IMT discriminates between patients with and without major vascular events and its association with the presence and number of major atherosclerosis risk factors. Maximal- and Mean-Maximal IMT were assessed by B-Mode ultrasonography and measured with an electronic caliper. Also by using this simple method of measurement, IMT values of the carotid artery were significantly greater in men than in women and were directly related to age, SBP, TC, LDL-C, TG, blood glucose and uric acid and inversely with HDL-C. Among risk factors, IMT was one of the best discriminants between patients with and without major vascular events. The IMT was also linearly related to the number of vascular risk factors, either in the whole group, or after stratification into age classes. These observations establish the predictive validity of IMT measured with the electronic caliper in large scale epidemiological studies and for the screening of individuals at high risk.

CORRELATION BETWEEN CELL PROLIFERATION, CHOLESTEROL METABOLISM AND MULTIDRUG RESISTANCE GENE EXPRESSION IN CEM AND MOLT4 LYMPHOBLASTIC CELLS.

B. Batetta, S. Sanna, RR Bonatesta, F. Mulas, S. Piras, M. Putzolu, A. Pani, O. Spano and S. Dessì. Istituto di Patologia Sperimentale, Università degli Studi di Cagliari, Cagliari (Italy).

Our previous studies on cholesterol metabolism in two cell lines CEM and MOLT4 isolated from patients with lymphoblastic T leukemia have shown that, although cholesterol synthesis is higher in MOLT4, the rate of growth is faster in CEM cells. CEM, however, exhibit a higher rate of cholesterol esterification suggesting that cholesterol esterification may be a mechanism whereby cells may regulate the rate of their growth and division. Under normal conditions, the rate of cholesterol esterification is not limited by ACAT levels but rather by the availability of cholesterol substrate in the endoplasmic reticulum (RE). Recently, the multidrug resistance (MDR) p-glycoproteins have been implied in this transport. To provide new insight into regulation of ACAT pathway during cell proliferation in the present study, the expression of MDR1, ACAT and other genes involved in the regulation of cholesterol metabolism such as HMGCoA reductase and LDL receptor were investigated during the growth of CEM and MOLT4 cell lines. The results have shown that the more rapid growing CEM cells had consistent lower levels of expression of HMGCoA-reductase and LDL receptors compared to MOLT4 suggesting that there is no apparent correlation between the rate of cholesterol synthesis and uptake and the growth capacity of these two cell lines. In contrast ACAT gene expression appears to be positively correlated with cell growth, this gene is more expressed in CEM cells, further supporting the concept that cholesterol esterification may have a role in modulating the rate of cell growth and division. The higher levels of ACAT gene expression in CEM were associated with higher levels of MDR1 gene expression, suggesting that the amount of the MDR1 gene product, by modulating intracellular cholesterol ester levels, may influence the rate of cellular growth.