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POSTPRANDIAL EFFECTS OF WINE CONSUMPTION ON LIPIDS AND OXIDATIVE STRESS BIOMARKERS

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Summary: *Postprandial lipemia has been recognized as a risk factor for atherosclerosis development. Consuming meals with suitable sources of antioxidants such as red wine reduces postprandial oxidative stress. However, information about the postprandial effects of wine ingestion outside meals on lipids and on* in vivo *low-density lipoprotein (LDL) oxidation in humans is scarce. The aim of this study was to investigate postprandial changes in lipids and* in vivo *LDL oxidation after moderate (250 ml) red wine ingestion, before and after sustained wine consumption of 250 ml/day for 4 days. After 4 days of sustained wine consumption a decrease in the LDL/high-density lipoprotein cholesterol ratio was observed after wine ingestion (p = 0.026). On day 4, a decrease in oxidized LDL levels and an increase in the antioxidant enzyme glutathione peroxidase activity (p = 0.025) were observed after wine ingestion. Our results show that consumption of red wine at moderate doses outside meals does not promote oxidative stress. Daily consumption of moderate doses of red wine can improve postprandial lipid profile and oxidative status when wine is ingested outside meals.*

Introduction

Postprandial lipemia has been recognized as a risk factor for atherosclerosis development as it is associated with oxidative changes, including an increase in triglyceride (TG)-rich lipoproteins and their remnants (1, 2). Meals, especially those high in fat, induce oxidative stress with impairment of endothelial function (3-6). However, consuming fatty meals with suitable sources of antioxidants such as red wine (4), vitamin C (5), or antioxidant drugs such as simvastatin (3) reduces postprandial oxidative stress.

Postprandial low-density lipoprotein (LDL) after a test meal was less resistant to *in vitro* oxidation than

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at baseline. The same meal with wine reduced postpandrial lipid peroxidation in healthy subjects (4). In diabetic patients, consumption of 300 ml of red wine during a meal counterbalanced the decrease in the susceptibility of LDL to oxidation and the thrombosis activation promoted by the meal (6). Thus, red wine consumption during a meal seems to have a beneficial effect in reducing meal-induced oxidative stress. However, information on the postprandial effects of wine ingestion alone on lipids and on *in vivo* LDL oxidation in humans is scarce.

The lifestyle in European Mediterranean countries has undergone changes in the last few decades (7): whereas wine consumption has decreased, consumption of beer and other types of alcohol, mainly distillates, has increased. Trends in self-reported past alcoholic beverage consumption and ethanol intake from 1950 to 1995, observed in eight European countries (8), indicate the existence of a geographical pattern. Wine consumption was observed to decrease in the time period evaluated in southern Europe and to increase in northern Europe, with an overall increase in beer consumption. Previous results based on analysis of per capita consumption data for Europe had reported adoption of wine consumption in the north and beer consumption in the south (9, 10). Alcohol in the United States and northern European countries is consumed outside mealtimes, especially at weekends (8, 11). Drinking large amounts outside mealtimes may be particularly harmful because the alcohol is rapidly absorbed and metabolizing enzymes are quickly saturated. Consumption of wine, the traditional beverage of the Mediterranean countries, in moderate doses has been associated with a decreased risk of coronary heart disease (12, 13). However, a study with pooled data from several Italian epidemiological studies found that drinking wine outside mealtimes was related to increased mortality from all causes (14). Thus, the effects of wine consumption outside meals merits investigation.

The aim of this study was to investigate postprandial changes in lipids and *in vivo* LDL oxidation after moderate doses (250 ml) of red wine ingestion, before and after sustained wine consumption of 250 ml/day for 4 days.

Material and methods

Subjects and study design. Ten healthy male nonsmoking volunteers were recruited. The mean age was 21.1 years (range: 20-22 years) and the mean body mass index was 20.9 (range: 20.6-26.3 kg/m^2). Subjects were considered healthy on the basis of physical examination and routine biochemical and hematological laboratory determinations. Volunteers followed a strict antioxidant-low diet for 3 days (washout period) before intervention periods. A nutritionist instructed them on the exclusion of several foods, rich in phenolic compounds, from their diet (vegetables, legumes, fruits, juices, wine, coffee, tea, cola, beer, cacao, jams, olives and olive oil). Daily dietary records were obtained from each volunteer. The same low phenolic diet was followed during the intervention period (4 days) in which meals were served in the center. On days 1 and 4 a single dose of 250 ml of red wine (total phenolic compound content = 3,250 mg/l gallic acid equivalents) was administered to the volunteers in the morning, in a fasting state. On days 2 and 3 the subjects received the same dose of wine per day but distributed among meals. Blood samples were collected at baseline and at 1 and 4 h after a single dose of 250 ml of wine.

Laboratory measurements. Serum glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were determined by enzymatic methods. LDL was calculated by Fridewald's formulae. Plasma-oxidized LDL (LDLox), as a marker of *in vivo* oxidation, was measured by an enzyme-linked

immunosorbent assay procedure using the murine monoclonal antibody mAb-4E6 as capture antibody bound to microtitration wells and a peroxidase conjugated antiapolipoprotein B antibody recognizing LDLox bound to the solid phase. Plasma glutathione peroxidase (GSH-Px) activity, as a marker of the endogenous antioxidant defenses, was measured by a modification of the Paglia and Valentine method (Ransel RS 505, Randox Lab., Crumlin, Northern Ireland) (15).

Statistical analyses. The normality of the distribution of the variables was assessed by the Kolmogorov-Smirnov test and by analysis of skewness and kurtosis. Logarithmic transformation was performed to normalize nonparametric variables (*i.e.* serum triglycerides). A general linear model for repeated measurements was fit, with multiple paired comparisons corrected by Tukey's method, in order to assess differences among evaluated times for each variable. Statistical significance was defined as $p < 0.05$. SPSS statistical software was used.

Results

After ingestion of a single dose of wine (250 ml), on days 1 and 4, a nonsignificant increase in serum triglycerides, together with a decrease ($p = 0.001$) in glucose levels, were observed (data not shown). No changes in the absolute values of LDL or HDL cholesterol after a single wine dose were observed on days 1 or 4. However, when the LDL/HDL cholesterol ratio was evaluated, a decrease in this ratio was observed from baseline to 4 h after a single dose of wine on day 4, after 4 days of sustained wine consumption ($p = 0.026$) (Fig. 1). Concerning *in vivo* measurements of lipid oxidation, a decrease in plasma oxidized LDL, with borderline significance ($p = 0.059$), was observed at 1 h after wine ingestion on day 1. On day 4, a significant linear decrease in oxi-

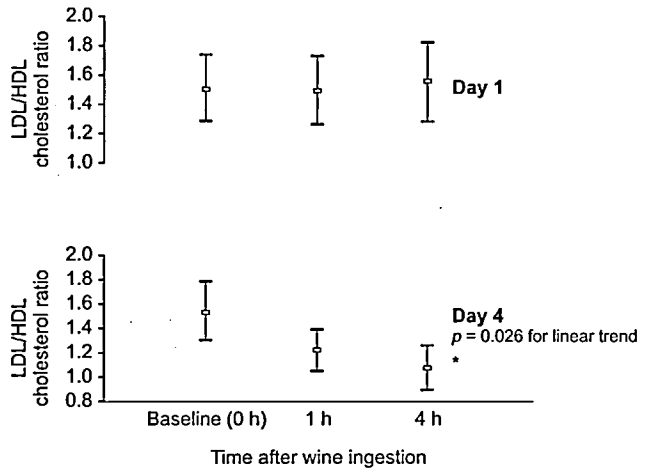

Fig. 1 Postprandial changes in the low-density lipoprotein (LDL)/high-density lipoprotein (HDL) cholesterol ratio after a single dose of wine (250 ml) in healthy male volunteers ($n = 10$) before (day 1) and after sustained (250 ml/day) wine consumption (day 4). *$p < 0.05$ *versus* baseline, Tukey's test.

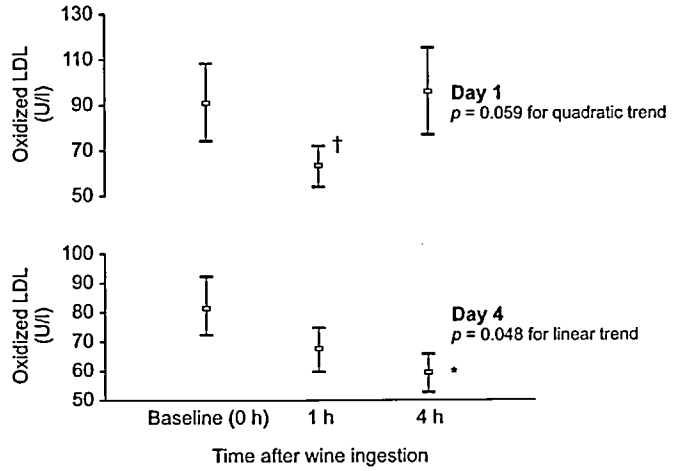

Fig. 2 Postprandial changes in *in vivo* oxidized low-density lipoprotein (LDL) after a single dose of wine (250 ml) in healthy male volunteers (n = 10) before (day 1) and after sustained (250 ml/day) wine consumption (day 4). *p < 0.05, †p < 0.06 *versus* baseline, Tukey's test.

dized LDL levels was observed from baseline to 4 h after wine ingestion (p = 0.048) (Fig. 2). Changes in GSH-Px activity showed opposite trends on days 1 and 4 at 1 h after wine ingestion. On day 1 a decrease, with borderline significance (p = 0.060), was observed at this point, whereas on day 4 a significant increase in antioxidant enzyme activity from baseline (p = 0.025) was observed (Fig. 3). Figure 4 summa-

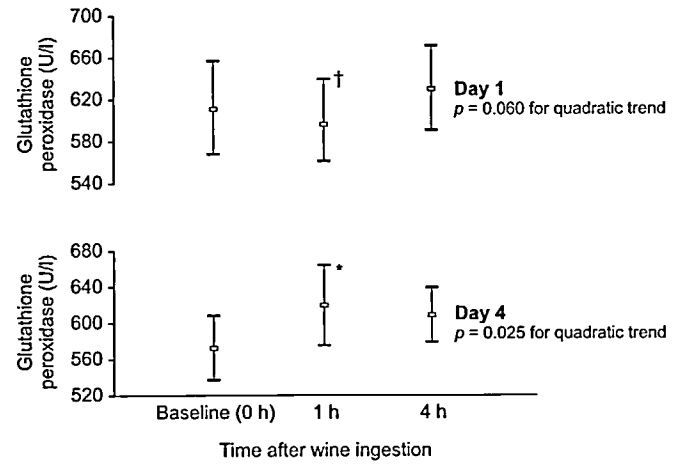

Fig. 3 Postprandial changes in glutathione peroxidase (GSH-Px) activity after a single dose of wine (250 ml) in healthy male volunteers (n = 10) before (day 1) and after sustained (250 ml/day) wine consumption (day 4). *p < 0.05 *versus* baseline, †p < 0.05 *versus* 4 h, Tukey's test.

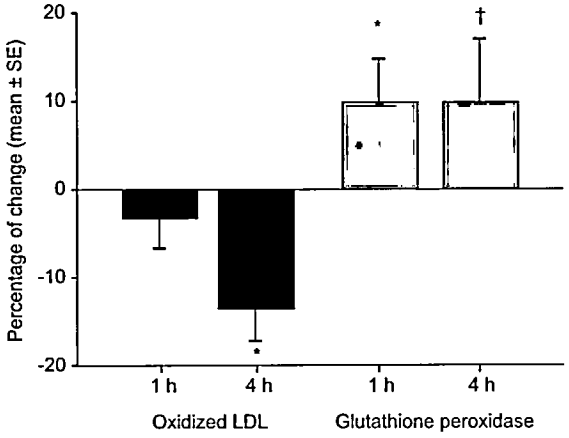

Fig. 4 Percentage of change in postprandial oxidative and antioxidative markers after a single dose of wine (250 ml) at day 4, after sustained (250 ml/day) wine consumption. *$p < 0.05$, †$p < 0.06$ *versus* baseline, Tukey's test. LDL = low-density lipoprotein.

rizes the percentage of change in postprandial oxidative and antioxidative markers on day 4. In addition to the decrease in oxidized LDL, an increase in GSH-Px was observed ($p < 0.05$).

Discussion

In this study postprandial changes in lipids and oxidative stress markers (*in vivo* LDL oxidation and the antioxidant enzyme GSH-Px activity) were examined after 250 ml of red wine ingestion before and after sustained wine consumption of 250 ml/day for 4 days. We did not observe postprandial oxidative stress. After 4 days of sustained wine consumption, a postprandial decrease in the LDL/HDL cholesterol ratio and in oxidized LDL levels together with an increase in GSH-Px were observed after a single dose of red wine (250 ml).

Several studies have examined the acute effects of wine ingestion on the serum lipid profile. In these studies, however, wine was consumed with meals and the effect of wine alone was not examined. Van Tol *et al.* (16) reported that daily consumption of 40 g of alcohol (beer, wine or spirits) with dinner for 4 weeks increased postprandial triglyceride levels and decreased postprandial LDL cholesterol in comparison with mineral water. The capacity of alcohol to raise HDL cholesterol levels is well known (17, 18). A postprandial increase in HDL cholesterol after alcohol consumption has recently been described (19). However, there is now increasing evidence of an effect of the phenolic compounds in raising HDL-cholesterol. In animal studies Mangas-Cruz *et al.* (20) showed that HDL cholesterol levels increased with olive oil enriched with phenolic compounds and decreased with olive oil impoverished in phenolic compounds. In other animal and human studies, an HDL-cholesterol–raising effect after the administration of polyphenol-rich plant extracts or juices has also been reported (22, 23). The separate effects of both alcohol and phenolic compounds of wine merit further investigation.

After meals, especially those rich in fat, oxidative stress occurs (3-6). An increase in lipid peroxidation,

together with a decrease in antioxidant defenses, is a common feature after acute oxidative stress such as that occurring with diabetes or acute exercise (24-26). Concerning dietary interventions, oxidative stress has been reported, reflected in an increase in plasma and very-low-density lipoprotein lipid peroxides and in a decrease in plasma GSH-Px and glutathione reductase (27), after ingestion of 50 ml of virgin olive oil, without changes in the resistance of LDL to oxidation.

In the present study, a slight decrease in both oxidized LDL and GSH-Px 1 h after wine ingestion on day 1 was observed. These changes suggest that GSH-Px activity was involved in the decrease of oxidized LDL. GSH-Px is a selenium-containing enzyme requiring glutathione. In addition to removing reactive oxygen species, specifically hydrogen peroxide, this enzyme detoxifies lipid peroxides to nontoxic alcohols, thus acting as a chain-breaking antioxidant of lipid peroxidation (28). After 4 days of sustained and moderate wine consumption (250 ml/day) we observed opposite trends in postprandial oxidative and antioxidative markers after a single dose of 250 ml of wine: a decrease in oxidized LDL and an increase in GSH-Px activity. The decrease in oxidized LDL suggests that antioxidants are involved in free radical scavenging, thus protecting LDL from oxidation. However, endogenous antioxidant defenses do not seem to be involved; if they were, we would have observed a decrease in GSH-Px as a consequence of its inactivation. In fact, there was a preservation of these antioxidant defenses which was reflected in the increase of GSH-Px activity. Given that red wine polyphenols were the only source of exogenous antioxidants for 3 days before and during the intervention period, our results suggest a role of wine polyphenols in the protection of LDL oxidation after wine ingestion.

The finding that the main changes in oxidative stress markers were observed after 4 days of wine consumption suggests phenolic compound accumulation from wine in the body on a short-term basis. Lamuela-Raventós *et al.* (29) demonstrated that individual dietary phenolic compounds, some of them present in wine, such as quercetine-3-glucuronide and flavonoids, can bind human LDL *in vivo* in non-supplemented individuals. Tyrosol, a phenolic compound present in white wine and virgin olive oil, has been shown to be capable of binding human LDL in *ex vivo* experiments (30). After red wine consumption, catechin and quercetin have been detected and quantified in human plasma (31) and in the LDL of apolipoprotein-deficient mice (32). Phenolic compounds that could bind LDL are likely to exert their peroxyl scavenging activity in the arterial intima, where oxidation of LDL preferentially occurs in microdomains sequestered from plasma antioxidants (33), thus avoiding the development of atherosclerosis.

In conclusion, consumption of red wine in moderate doses and taken outside meals does not promote oxidative stress. Daily consumption of moderate doses of red wine can improve postprandial lipid profile and oxidative status when wine is ingested outside meals. Our results support a role for wine phenolics in postprandial protection against LDL oxidation after daily sustained and moderate doses of wine ingestion.

References

(1) Roche H.M., Gibney M.J. *The impact of postprandial lipemia in accelerating atherothrombosis.* J. Cardiovasc. Risk, **7**, 317, 2000.

(2) Regnstrom J., Nilsson J., Tornvall P., Landou C., Hamsten A. *Susceptibility to low-density lipoprotein oxidation and coronary atherosclerosis in man.* Lancet, **339**, 1183, 1992.

(3) Ceriello A., Taboga C., Tonutti L., et al. *Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation: Effects of short- and long-term simvastatin treatment.* Circulation, **106**, 1211, 2002.

(4) Natella F., Ghiselli A., Guidi A., Ursini F., Scaccini C. *Red wine mitigates the postprandial increase of LDL susceptibility to oxidation.* Free Radic. Biol. Med., **30**, 1036, 2001.

(5) Ling L., Zhao S.P., Gao M., Zhou Q.C., Li Y.L., Xia B. *Vitamin C preserves endothelial function in patients with coronary heart disease after a high-fat meal.* Clin. Cardiol., **25**, 219, 2002.

(6) Ceriello A., Bortolotti N., Motz E., et al. *Red wine protects diabetic patients from meal-induced oxidative stress and thrombosis activation: A pleasant approach to the prevention of cardiovascular disease in diabetes.* Eur. J. Clin. Invest., **31**, 1365, 2001.

(7) De la Torre-Boronat M.C. *Scientific basis for the health benefits of the Mediterranean Diet.* Drugs Exp. Clin. Res., XVX (2/3), 155, 1999.

(8) Klipstein-Grobusch K., Slimani N., Krogh V., et al. *Trends in self-reported past alcoholic beverage consumption and ethanol intake from 1950 to 1995 observed in eight European countries participating in the European Investigation into Cancer and Nutrition (EPIC).* Publ. Health. Nutr., **5**, 1297, 2002.

(9) Hupkens C.L., Knibbe R.A., Drop M.J. *Alcohol consumption in the European community: Uniformity and diversity in drinking patterns.* Addiction, **88**, 1391, 1993.

(10) Norström T. *Alcohol in postwar Europe: Consumption and drinking patterns, consequences and policy responses in 15 European countries*. Stockholm: European Comission, 2001.

(11) Mukamal K.J., Conigrave K.M., Mittleman M.A., et al. *Roles of drinking pattern and type of alcohol consumed in coronary heart disease in men*. New Eng. J. Med., **348**, 109, 2003.

(12) St. Leger A.S., Cochrane A.L., Moore F. *Factors associated with cardiac mortality in developed countries with particular reference to the consumption of wine.* Lancet, **1**, 1017, 1979.

(13) Gronbaek M., Deis T.I.A., Sorensen H., et al. *Mortality associated with moderate intake of wine, beer, or spirits.* BMJ, **310**, 1165, 1995.

(14) Trevisan M., Schisterman E., Mennotti A., Farchi G., Conti S. *Drinking pattern and mortality: The Italian risk factor and life expectancy pooling project.* Ann. Epidemiol., **11**, 312, 2001.

(15) Paglia D.E., Valentine W.N. *Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase*. J. Lab. Clin. Med., **70**, 158, 1967.

(16) Van Tol A., van der Gaag M.S., Scheek L.M., van Gent T., Hendriks H.F. *Changes in postprandial lipoproteins of low and high density caused by moderate alcohol consumption with dinner.* Atherosclerosis, **141**, 101, 1998.

(17) Suh I., Shaten B.J., Cutler J.A., Kuller L.H. *Alcohol use and mortality from coronary heart disease: The role of high-density lipoprotein cholesterol.* Ann. Inter. Med., **1**, 881, 1992.

(18) Gaziano J.M., Buring J.E., Breslow J.L., et al. *Moderate alcohol intake, increased levels of high-density lipoprotein and its subfractions, and decreased risk of myocardial infarction.* Engl. J. Med., **16**, 1829, 1993.

(19) Chung B.H., Doran S., Liang P. *Alcohol mediated enhancement of postprandial lipemia: A contributing factor to an increase in plasma HDL and a decrease in risk of cardiovascular disease.* Am. J. Clin. Nutr., **78**, 391, 2003.

(20) Mangas-Cruz M.A., Fernández-Moyano A., Albi T., et al. *Effects of minor constituents (non-glyceride compounds) of virgin olive oil on plasma lipid concentrations in male Wistar rats.* Clin. Nutr., **20**, 211, 2001.

(21) Kurowska E.M., Spence J.D., Jordan J., Wetmore S., Freeman D.J., Piché L., Serratore P. *HDL-cholesterol-raising effect of orange juice in subjects with hypercholesterolemia.* Am. J. Clin. Nutr., **72**, 1095, 2000.

(22) Ebrahim Sajjadi S., Movahedian Atar A., Yektaian A. *Antihyperlipidemic effect of hydroalcoholic extract, and polyphenolic fraction from Dracocephalum kotschyi Boiss.* Pharm. Acta Helv., **73**(3), 167, 1998.

(23) Ruíz C., Alegría A., Barbera R., Farre, R., Lagarda M.J. *Lipid peroxidation and antioxidant enzyme activities in patients with type 1 diabetes mellitus.* Scand. J. Clin. Lab. Invest., **59**, 99, 1999.

(24) Efe H., Deger O., Kirci D., Karahan S.C., Orem A., Calapoglu M. *Decreased neutrophil antioxidative enzyme activities and increased lipid peroxidation in hyperlipoproteinemic human subjects*. Clin. Chim. Acta., **279**, 155, 1999.

(25) Sen C.K. *Oxidants and antioxidants in exercise*. Am. J. Physiol., **30**, 675, 1995.

(26) Elosua R., Molina L., Fito M., et al. *Response of oxidative stress biomarkers to a 16-week aerobic physical activity program, and to acute physical activity, in healthy young men and women.* Atherosclerosis, **167**, 327, 2003.

(27) Fitó M., Gimeno E., Covas M.I. *Postprandial and short-term dietary intervention effects of virgin olive oil ingestion on the oxidative/antioxidative status*. Lipids, **37**, 245, 2002.

(28) Gutteridge J.M. *Lipid peroxidation and antioxidants as biomarkers of tissue damage*. Clin. Chem., **41**, 1819, 1995.

(29) Lamuela-Raventós R.M., Covas M.I., Fitó M., Marrugat J., De la Torre-Boronat M.C. *Detection of dietary antioxidant phenolic compounds in human LDL.* Clin. Chem., **45**, 1870, 1999.

(30) Covas M.I., Fitó M., Lamuela-Raventós R.M., Sebastiá, N., de la Torre-Boronat C., Marrugat J. *Virgin olive oil phenolic compounds: Binding to human LDL and effect on LDL oxidation.* Int. J. Clin. Pharm. Res., XX(3/4), 49, 2000.

(31) Donovan J.L., Bell J.R., Kasim-Karakas S., et al. *Catechin is present as metabolites in human plasma after consumption of red wine.* J. Nutr., **129**, 1662, 1999.

(32) Aviram M., Furhman B. *Wine flavonoids protect against LDL oxidation and atherosclerosis*. Ann. N. Y. Acad. Sci., **957**, 146, 2002.

(33) Witzum J.L. *The oxidation hypothesis of atherosclerosis*. Lancet, **1344**, 793, 1994.