

Abstracts to the First Seminar “Responsible drinking within the Mediterranean diet and Italian lifestyle”, June 10–11, 2011, Pontignano, Italy

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Foreword

The scientific seminar “*Responsible drinking within the Mediterranean diet and Italian lifestyle*” was conceived as an attempt to put together most Italian research teams dealing with beneficial health effects of moderate alcohol consumption, and in particular wine. The initiative was born from a fruitful collaboration between the “Umberto Pallotta Vino e Salute” Group of the Italian Academy of Grapevine and wine, the Academy of Georgofili, the Catholic University of Campobasso, the Universities of Ancona and Siena with the support of the Enoteca Nazionale di Siena and Federvini and under the patronage of the Italian Ministries of Agriculture and Health and of the Italian Society of Human Nutrition.

The meeting was held in June 10th and 11th 2011 in the ancient Abbey of Pontignano, near Siena, in the heart of the wine-growing zones of Tuscany. It had been officially presented, a few weeks before, by the Nobel prize for Medicine, Louis J. Ignarro, during the opening ceremony of the academic year of the Academy of Georgofili in Florence, where the Italian-American scientist stressed the importance of reinforcing the health-promoting role of antioxidants in the diet.

The main purpose of the seminar in Pontignano was to focus on the most recent evidence-based Italian research on the effects of moderate consumption of wine and alcoholic beverages within the Mediterranean Diet, a characteristic Italian lifestyle. The seminar was timely in a moment in which alcoholics are at the center of an international offensive strategy, condemning all kinds and levels of alcoholic consumption, without considering the difference between moderate and hazardous drinking. Accordingly, in recent public health strategies or in the media, not just the heavy drinkers are blamed but also responsible drinkers, who regularly consume moderate amounts of alcohol during their meals. Moderate drinking, on the contrary, is a healthy habit, which has been fully promoted by science, with benefits confirmed by a large series of clinical and epidemiological studies worldwide.

The meeting in Pontignano highlighted ongoing studies conducted on this topic by Italian research groups and provided further evidence on the role of moderate drinking as a crucial issue in the prevention of cardiovascular disease.

Spanning from biochemistry to genetics and to population studies, the scientific programme dealt with the topic of the effects of alcohol and wine consumption, trying to provide a complete state of the art in the field. Most scientists who joined the seminar were deeply convinced that the Northern Europeans, and especially their youth, have serious problems with alcohol abuse also due to binge-drinking habits. However, the situation is quite different in Southern areas of Europe, where alcoholic drinks and wine are mostly not a tool to get drunk, but still represent a cornerstone of the Mediterranean diet, a healthy lifestyle which was also recently inscribed on the list of Human Intangible Heritage by UNESCO.

What came out from the meeting in Tuscany? A loud and documented NO to public bans (with no strings attached) against alcohol and a determined YES to science and to the value of the Mediterranean way when it comes to drinking.

The Organisers wish to thank the European Journal of Nutrition for the opportunity to publish the abstracts presented at the Scientific Seminar “Responsible drinking within the Mediterranean diet and the Italian lifestyle”, and are grateful to all the Speakers and Authors who contributed to the success of the initiative.

Special thanks go to Marialaura Bonaccio of the Catholic University of Campobasso, Italy, for the competent and enthusiastic contribution to the success of the meeting and to the publication of these abstracts.

This publication is based on the First Seminar “Responsible drinking within the Mediterranean diet and Italian lifestyle” held on June 10–11, 2011 in Pontignano, Italy. This meeting was sponsored by the “Umberto Pallotta Vino e Salute” Group of the Italian Academy of Grapevine and wine, the Academy of Georgofili, the Catholic University of Campobasso, the Universities of Ancona and Siena and partially financed by the Enoteca Nazionale di Siena and Federvini, and was under the patronage of the Italian Ministries of Agriculture and Health and of the Italian Society of Human Nutrition.

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Acute effects of low doses of red wine on cardiac function: echocardiographic analysis in healthy subjects

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Purpose: Moderate-to-high blood concentrations of ethanol acutely impair left ventricular (LV) performance, but the effects of low concentrations are unclear. This study explored the acute effects of low blood concentrations of ethanol on sensitive and load-independent indices of LV and right ventricular (RV) function.

Methods: Forty nine young volunteers drank a light-to-moderate quantity of red wine (5 ml/kg). As a control, after few days, the same population drank the same volume of an alcohol-free juice. The following measurements were performed, both at baseline and 60 min after the challenges, using tissue Doppler echocardiography: mitral annulus systolic velocity (S') and excursion (MAPSE), early diastolic velocity (E'), and its ratio to late diastolic velocity (E'/A'); tricuspid annulus systolic velocity (tricuspid S') and excursion (TAPSE), early diastolic velocity (tricuspid E'), and its ratio to late diastolic velocity (tricuspid E'/A').

Results: Compared to the control challenge, ethanol induced a % decrease in all measures of LV function (S' , -9.7%; E' , -11.2%; E'/A' , -13.4%; MAPSE, - 8.8%; $p < 0.05$ for all parameters). Among RV function, increases in tricuspid E'/A' ratio and TAPSE were observed (+24.5% and +9.0%, respectively; $p < 0.05$ for both).

Conclusions: Low blood concentrations of ethanol acutely impair LV function and increase some indices of RV function in young healthy individuals.

Keywords: red wine, echocardiography, tissue Doppler.

Moderate wine or beer drinking equally protects against fatal and non-fatal cardiovascular events: a meta-analysis

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Purpose: Epidemiologic studies indicate that moderate alcohol consumption protects against cardiovascular disease (1,2). We performed an updated meta-analysis on the relationship between either wine or beer consumption and fatal and non-fatal cardiovascular risk.

Methods: Articles were retrieved through March 2011 by search in PubMed and EMBASE. A weighed, least-squares regression analysis of second-order fractional polynomial models was applied to pooled data derived from studies that gave quantitative estimation of the cardiovascular risk associated with either wine or beer consumption.

Results: From 16 pooled studies there was evidence supporting a J-shaped relationship between increasing amounts of wine intake and cardiovascular risk (maximal protection: 31% (95% CI: 19–42%) at 21 g/d of alcohol (2 glasses of wine). Similarly, from 13 pooled studies, an inverse J-shaped relationship was also apparent between increasing amounts of beer intake and cardiovascular risk (maximal protection: 42% (95% CI: 19–58%) at 43 g/d of alcohol (3 cans of beer)). From studies reporting separate data obtained both from wine and beer

consumers, two closely overlapping curves were obtained (maximal protection: 36% at 25 g/day of alcohol by either beverage).

Conclusions: This meta-analysis gives evidence for a J-shaped association between either wine or beer consumption and cardiovascular risk. Dose-response curves appeared substantially similar for both alcoholic beverage consumption, suggesting that, in relation to health, the amount and the way of drinking are more important than the content of the bottle.

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Keywords: alcohol, beer consumption, wine consumption, cardiovascular disease, meta-analysis

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Alcohol intake and hemochrome parameters in the healthy population of the MOLI-SANI study

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Purpose: To evaluate the association of platelet count, mean platelet volume (MPV), white blood cells (WBC), red blood cells (RBC), lymphocyte, monocyte and granulocyte counts with alcohol intake.

Methods: Healthy subjects from the “Moli-sani” study (a population-based cohort of 24,318 Italians ≥ 35 years) were analyzed ($n = 18,531$; 51% women, mean \pm SD age: 56 ± 12 years). Hemochromocytometric analyses were performed using an automatic analyzer (Beckman Coulter, model IL, Milan, Italy). Alcohol consumption was evaluated using a specific validated questionnaire. Values are means \pm standard deviation.

Results: Women: Average platelet counts were 260 ± 65 10⁹/L in moderate (0–12 g/day), 266 ± 66 in abstainers and 264 ± 65 in heavy drinkers (> 24 g/day); these differences were significant ($P = 0.0053$) after adjusting for age, BMI, total caloric intake, cigarette smoking and social status. In the same three groups MPV were 8.99 ± 1.1 femtoliters (fL), 9.23 ± 1.3 and 9.10 ± 1.2 ; ($P < 0.0001$) and monocytes 6.5 ± 1.9 %, 6.9 ± 2.0 and 6.8 ± 1.9 ($P < 0.0001$). RBC decreased increasing alcohol intake (4.73 ± 0.40 10³/ μ L, 4.70 ± 0.39 and 4.66 ± 0.39 ; ($P < 0.0001$).

Men: Average monocyte count (\pm SD) were 7.2 ± 2.1 %, 7.4 ± 2.1 and 7.6 ± 2.3 ($P < 0.0001$) in moderate (0–24 g/day) abstainers and heavy drinkers (> 48 gr/day). MPV was 9.19 ± 1.4 fL, 8.87 ± 1.2 and 8.81 ± 1.2 ($P < 0.0001$) and WBC 6.5 ± 2.2 10³/ μ L, 6.4 ± 2.0 and 6.3 ± 1.6 ($P = 0.014$) in the same groups, respectively. RBC decreased increasing alcohol intake (5.23 ± 0.45 10³/ μ L, 5.21 ± 0.47 and 5.09 ± 0.43 ($P < 0.0001$).

Similar results were found taking into account wine instead of alcohol consumption.

Conclusions: Moderate alcohol consumption was associated with significant variations of hemochrome parameters, supporting its role in the prevention of cardiovascular disease.

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Keywords: alcohol intake, cardiovascular diseases, hemochrome parameters, inflammation

Effects of alcohol-free wine on DNA oxidation damage and gene expression in rodents

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Background and purpose: Polyphenols are important constituents of a variety of foods, with supposed health-promoting properties. Polyphenols are contained at relatively high concentrations in wine, but understanding their action is partially complicated by the alcohol content, which has pharmacological and toxicological activity on its own. Therefore, we decided to investigate the effect of alcohol-free wines in chronic feeding experiments in rodents. Moreover, the action of polyphenols on oxidation damage has received a considerable attention, whereas the effects on gene regulation *in vivo* are still poorly characterized.

Methods: Wistar rats were fed the AIN76 diet, modified to mimic high-fat human diets and, as a source of low and high dietary polyphenols, we used white and red alcohol-free wines, prepared by the Experimental Unit Pech Rouge, INRA, Montpellier, France. Alcohol-free wines were administered for 4 weeks, replacing water as a drinking fluid (estimated exposure to tannins and flavanols was 7 and 55 mg/kg/d for white and red alcohol-free wine, respectively). At sacrifice, brain, intestine, liver and blood were obtained for the determination of oxidation damage, using the comet assay, and for gene expression profiling (liver and colon mucosa) employing home-made oligo arrays, containing probes for about 6,000 rat genes.

Results: No major differences in DNA damage were found in different organs between rats fed alcohol-free wines compared to controls; however, we observed a reduction in DNA oxidative damage at the level of the liver and of the *corpus striatum*. In the colon mucosa and the liver many genes were down-regulated by alcohol-free red wine and a lower number by alcohol-free white wine. Most notably, in the colon mucosa genes of prostaglandin synthesis (9 of 19 measured) and cytokines inflammation response (14 out of 20) were down-regulated by alcohol-free red wine.

Conclusions: Polyphenols of red and white wine have some effects on oxidation damage and marked effects on gene expression, both in the gastro-intestinal tract and in the periphery. These last effects

might arise directly from absorbed phenols or their metabolites or be mediated by variations of the intestinal flora, which in itself is considerably influenced by the consumption of wine polyphenols.

Keywords: alcohol free wine, polyphenols, gene expression, oxidation damage

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Vascular ion channels as targets of wine polyphenols and their metabolites: a possible explanation for their beneficial health effects

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Purpose: Recently, interest in wine polyphenols has greatly increased owing to their possible beneficial effects in cardiovascular disease. Since wine polyphenols can affect vessel tone, the aim of the present investigation was to verify whether vascular ion channels are targets for quercetin-like flavonoids.

Methods: Twenty four flavonoids were analysed for their effects on Cav1.2 (ICa1.2) or Cav3.1 channel current (ICa3.1) as well as KCa1.1 channel current in rat tail artery myocytes, using the whole-cell patch-clamp method.

Results: Most flavonoids either stimulated or inhibited ICa1.2 in a concentration-dependent manner with EC50/IC50 values in the μ M range. Key structural requirements for ICa1.2 modulatory activity were revealed by molecular modelling techniques. Galangin and chrysin were the only ICa3.1 inhibitors also capable of antagonising ICa1.2. (\pm)-Naringenin [1] and quercetin were able to stimulate KCa1.1 channel current. Cardamonin behaved like a bifunctional vasodilator capable of inhibiting ICa1.2 and stimulating KCa1.1 current [2]. Finally, a combination of quercetin metabolites, namely isorhamnetin, tamarixetin, and kaempferol, reduced ICa1.2 in the 1–100 nM concentration range.

Conclusions: Quercetin-like polyphenols behaved as vascular ion channel modulators. In the long term, dietary flavonoids, through the fine control of both KCa1.1 and Cav channels, might reduce vessel tone, thus producing beneficial antihypertensive effects.

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Keywords: quercetin, flavonoids, ion channels

Right atrial enlargement, P-wave extension, PR and QTc intervals prolongation after acute red wine assumption

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Purpose: Acute high alcohol consumption and new onset atrial fibrillation or other supra-ventricular and ventricular tachy-arrhythmias have been often associated. The aim of this study was to establish which alterations, assessed by echocardiography and electrocardiography, could be induced by acute red wine ingestion.

Methods: Fifty-two healthy young volunteers drank a light-to-moderate quantity of red wine (5 ml/kg). As a control, after few days, the same population drank the same volume of an alcohol-free juice. Echocardiographic exams and measurements of ECG time-intervals were performed both at baseline and after 60 min from the challenge. The mean blood ethanol concentration \pm SD after drinking was 0.49 ± 0.05 g/l.

Results: Compared to the control challenge, significant changes after red wine intake were observed in the right atrial area (mean values \pm SD): from 13.3 ± 2.8 to 15.5 ± 3.5 cm²; $p = 0.0003$, P wave duration (from 99 ± 11 to 108 ± 13 ms, $p < 0.0001$), PR interval (from 152 ± 14 to 167 ± 17 ms, $p < 0.0001$), QT interval (from 342 ± 27 to 360 ± 22 ms, $p < 0.0001$), and corrected QT (from 388 ± 24 to 403 ± 31 ms, $p = 0.0015$). In multivariate analyses for predictors of echo and ECG changes, none of the variables entered the final models.

Conclusions: Acute ingestion of red wine is associated with a significant increase of right atrial area and of most electrocardiographic time intervals in normal subjects. The potential arrhythmogenic impact of these effects is worth of further exploration.

Keywords: red wine, electrocardiography, echocardiography.

Red wine prevents the postprandial increase of plasma cholesterol oxidation products: a pilot study

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Purpose: Epidemiological studies report that moderate wine consumption can reduce cardiovascular risk. The control of postprandial hyperlipemia, a well-defined risk factor for atherosclerosis, is one of the possible mechanisms, reasonably through the reduction of the absorption of lipid-oxidized species from the meal. The aim of this study was to verify the hypothesis that wine consumption with meals is able to reduce the postprandial increase of plasma lipid hydroperoxides and cholesterol oxidation products in humans.

Methods: In two different sessions, 12 healthy volunteers consumed the same test meal rich in oxidized and oxidizable lipids (a double cheeseburger, total oxycholesterols 3.9 ± 1.1 μ g/g, mean \pm SD), in addition to 300 ml red wine or not. The postprandial plasma concentration of cholesterol oxidation products was measured by GC-MS.

Results: The control meal induced a significant increase ($p < 0.05$) of plasma concentration of lipid hydroperoxides, 7- β -hydroxycholesterol, and 7-ketocholesterol (+71%, +75% and +131%, respectively), two hours from time 0. Drinking red wine with the meal fully prevented these increases.

Conclusions: Consumption of wine with meals can prevent the postprandial increase of plasma cholesterol oxidation products. The possible mechanisms explaining this effect include: i) reducing lipid

peroxidation products or preventing their formation in the digestive tract; ii) delaying fat absorption; iii) inducing detoxifying enzymes in gut and liver; iii) enhancing the cholesterol oxidation products clearance; iiiii) chemically reducing lipid hydroperoxide and/or oxy-cholesterols into circulation after their absorption.

Keywords: cholesterol oxidation, wine

Human bioavailability of resveratrol from moderate consumption of red wine

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Background and purpose: Epidemiological studies associate moderate consumption of red wine with a reduced risk of CVD. Recent findings from human studies (1,2), indicate that metabolites from *in vivo* bio-conversion and microflora activity (rather than parental compounds) have a major role in the health benefits of dietary polyphenols. We wanted to clarify human bioavailability of resveratrol deriving from a moderate consumption of red wine.

Methods and results: Twenty five healthy subjects were enrolled and three experimental conditions were applied: consumption of red wine at fasting, associated with a standard meal, associated with meals with a different fat content. Free trans-resveratrol, 3-glucuronide- and 4'-glucuronide- resveratrol were quantified in serum samples, over 4 h period after wine consumption.

Free trans-resveratrol was found, in trace amounts, only in some serum samples collected 30 min after wine ingestion, while resveratrol-glucuronides predominated at following times. Trans-resveratrol bio-availability was independent from the type of meal or its fat content.

Conclusions: Our data suggest that the whole mixture of antioxidants in red wine and plasma increase of their metabolites may better explain epidemiological evidence than the level of parent resveratrol, a conclusion requiring additional human long-term studies.

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Keywords: resveratrol metabolites, red wine

The anticancer effects of resveratrol in glioma cells

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Purpose: Resveratrol (RESV), a natural constituent of wine and grapes, is widely studied both for its beneficial effects on human

health and for the role exerted in cancer prevention and therapy (1). Recently we have shown that RESV induces the expression of γ H2AX in human glioma cells treated *in vitro* and inhibits the catalytic activity of Topoisomerase II α (TOP2) (2). The aim of our study is to understand whether RESV can act as a Topo-poison, through the stabilization of the “cleavage complex” DNA-TOP2, so inducing DNA double strand breaks (DSBs).

Methods: We performed a docking simulation in order to show the putative complex between DNA, RESV and TOP2. An *In Vivo* Complex of Enzyme (ICE) assay was utilized to detect the persistence of covalent complexes between DNA and TOP2 in human glioma cells. The Cytokinesis Block Micronucleus assay (CBMN) was performed by a conventional method as a measure of DSBs.

Results: Docking simulation showed that RESV can interact both with DNA and RESV through several hydrogen bonds. The ICE assay confirmed that RESV (120 μ M) participates in the formation of covalent complexes DNA-TOP2 in glioma cells. RESV significantly ($p < 0.05$) increases micronuclei induction dose-dependently (80 μ M = 0.034 ± 0.017 vs 0.010 ± 0.003 ; 120 μ M = 0.062 ± 0.018 vs 0.010 ± 0.003 ; means \pm SD).

Conclusions: The confirmation of RESV as a TOPO-poison together with its ability to delay cell cycle suggests that this molecule may become an important anti-tumour agent against high grade gliomas, which are particularly resistant to conventional therapies.

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Keywords: resveratrol, topoisomerase II α , cleavage complex, micronuclei.

Individual behaviour response consequent to alcohol consumption: the role of genetic variability

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Background and aim: Young people are used to drink alcoholic beverages during weekend without any familiarity to this habit. Therefore, they experience different reactions, sometimes with dramatic consequences, possibly due to environmental and genetic factors. We have assessed the inter-individual variability in alcohol metabolism and its effect on car driving capabilities following acute alcohol intake.

Methods and results: About one hundred university students were recruited to assess their alcohol clearance and their virtual driving capability with a computer assisted equipment after the intake of an amount of wine adjusted for body surface area and gender. Alcohol clearance of a was assessed using an ethylometer, driving capability by assessing the reaction times before and after alcohol intake and individual genotype by saliva DNA analysis. Genetic polymorphisms of genes related to alcohol metabolism (ADH1C) and attention deficit disorders (ADHD: DAT1) were strongly associated with alcohol clearance and driving capability (measured as reaction time).

Conclusions: Interindividual genetic variability can account for a large part of variance among individuals in alcohol metabolism as well as in reaction times, suggesting that physiological tests are more suitable to assess car driving capability than the use of an ethylometer alone.

Keywords: genetic variability, alcohol intake, driving performance

Effect of colon-derived polyphenol metabolites on *in vitro* protein glycation

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Purpose: Polyphenols are thought to be beneficial in the control of diabetic complications because of their ability to inhibit oxidative stress and protein glycation [1]. Polyphenols undergo extensive metabolism during their absorption through the intestinal tract and their metabolites, produced by the colon microbiota can enter the circulatory system [2]. The aim of this study was to investigate the inhibitory activity on protein glycation of colonic flora-derived metabolites of polyphenols.

Methods: Protein glycation was measured, after a two week incubation *in vitro* under physiological conditions (pH 7.4, 37°C) of bovine serum albumin (40 mg/mL) and glucose (30 mmol/L) in the presence or absence of polyphenol metabolites, determining the variation of fluorescence at the excitation and emission of 355 and 405 nm, respectively.

Results: A dose-dependent inhibition of glycation was observed with urolithin A and B (metabolites of polyphenols from pomegranates and raspberries) and dihydroferulic acid (from coffee.) The IC₅₀ of urolithin A was 12 μ M. Urolithin B and dihydroferulic acid A were less effective. The polyphenol metabolites were also tested in association, based on their ratios in dietary sources. The pomegranate-derived metabolites urolithin A, B and pyrogallol, inhibited glycation 37% and 44% (1 and 2 μ mol/L, respectively), suggesting a synergic action between these metabolites. The red wine-derived metabolites showed a lower antiglycation activity (15% inhibition at 2 μ mol/L) while coffee metabolites were inactive.

Conclusions: Colon-derived metabolites of polyphenols inhibit protein glycation at concentrations that are similar to the ones usually observed in blood after consumption of a food portion of pomegranate or raspberry. Some polyphenols may therefore contribute to reduce hyperglycemia related to pathological conditions.

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Keywords: protein glycation, polyphenols metabolites, red wine, pomegranate juice

Polyphenols content, antioxidant activity and HPLC analysis of anthocyanins in grape, grape juices and musts

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Purpose: As alcohol-related disorders account for an enormous part of the global mortality and morbidity, the scientific research has been directed on alternative grape products, as a source of active polyphenols, involved in decreasing the risk factors for cardiovascular diseases. The aim of this study was to investigate and compare in parallel the antioxidant activity (AOA), the total polyphenol content (TPC) and the anthocyanin profiles of grape, musts (red, white and rosé), and full grape juices.

Methods: The antioxidant activity was measured spectrophotometrically at 517 nm after reaction with 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (5 mg/100 mL methanol).

Total polyphenol content was determined with the Folin-Ciocalteu colorimetric assay, measuring the absorbance at 765 nm. Anthocyanins (cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin) were analyzed by HPLC using a gradient elution, an inverse phase column (RP12) and a diode-array detector (DAD).

Results: Comparing data of antioxidant activity with the total polyphenol content and the anthocyanins HPLC profile, a good linear correlation can be observed. In all samples analysed, malvidin 3-O-glucoside resulted the most representative anthocyanin, and the highest levels were found in a red grape juice (132,04 +/- 2,87 mg/L SD).

Conclusions: HPLC anthocyanin profile showed a higher content of peonidin 3-O-glucoside (51,78 +/- 0,43 mg/L SD) and malvidin 3-O-glucoside in the fresh grape juices. Probably they are the most important compounds in determining the antioxidant activity of grape derivatives.

However, the antioxidant activity should be confirmed in human studies, in order to evaluate the bioavailability of the polyphenols of these beverages.

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Keywords: polyphenols, anthocyanins, antioxidant activity, HPLC.

Inducible defences elicited by a new chitosan formulation prevent powdery mildew infection in grapevine and melon

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Background and purpose: Elicitors can trigger plant defence when recognized by host receptors (1). Chitosan acts as a MAMP microbe-associated molecular patterns (MAMP) (2), eliciting systemic immunity.

In this study the inducible defences elicited by a new chitosan formulation (Valagro 5-1570) against powdery mildew infection was evaluated in both Trebbiano and Montepulciano grapevines. The effect of treatments on grapevine polyphenol profile was investigated. **Methods and results:** Grapevine plants (1999) were sprayed weekly with either Valagro 5-1570 or traditional fungicides. The percentage of infection was evaluated in leaves and bunches. The polyphenol profiles was investigated by HPLC at veraison and ripening.

All Valagro 5-1570 concentrations tested were effective in controlling powdery mildew, the best concentration being 10 ml l⁻¹. The polyphenol profiles of 5-1570 treated Trebbiano did not vary significantly but in Montepulciano treated berries flavonols (52,38 ± 47,62 ppm) and stilbenes (75,79 ± 8,79 ppm) increased significantly, in comparison with flavonols (293,41 ± 19,62 ppm) and stilbenes (9,22 ± 3,79 ppm) in untreated berries (averages ± SD, p < 0.05).

Conclusions: Valagro 5-1570 provides an efficient control of powdery mildew in grapevine. Applied weekly, the 10 ml l⁻¹ rate was as effective as traditional fungicides. This formulation acts as an inducer of defence responses and can be an alternative mean of disease control. Preliminary results of polyphenol content of grapes indicated for red grapes a beneficial effect of chitosan, increasing some important polyphenol classes, including stilbenes.

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Keywords: mildew infections, grapes, chitosan

Effect of different alcoholic beverages on gastric epithelial cell: role of oxidative stress

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Background and aims: Alcohol is known to damage the gastric epithelium. This study was designed to evaluate whether various alcoholic beverages might have different damaging effect on gastric epithelial cells *in vitro*.

Methods: Cell damage was assessed by MTT assay in MKN-28 gastric epithelial cells. Oxidative stress was induced by incubating cells with 1 mM xanthine and 50 mU ml⁻¹ xanthine oxidase. Gastric cell viability was assessed after 30, 60, and 120 min incubation with ethanol (17.5–125 mg/ml⁻¹) or with different alcoholic beverages (i.e., beer, white wine, red wine, spirits) at comparable ethanol concentration. Finally, we assessed whether pre-incubation with red wine (with or without ethanol) prevented oxidative stress-induced cell damage.

Results: Red wine caused less damage to gastric epithelial cells *in vitro* as compared with other alcoholic beverages; pre-treatment with red wine significantly and time-dependently prevented oxidative

stress-induced cell damage (45% viability without pre-treatment vs 65% and 70%, after 60 and 120 min of pre-incubation, respectively); pre-treatment with de-alcoholized wine did not prevent oxidative stress-induced damage to gastric cells.

Conclusions: Red wine was less harmful to gastric epithelial cells than other alcoholic beverages. This seems to be related to non-alcoholic components of red wine, since other alcoholic beverages used in the experiments had comparable ethanol concentration. Red wine prevents oxidative stress-induced cell damage, an effect apparently related to its ethanol content.

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Keywords: red wine, alcohol, gastric epithelial cells

Nephroprotective effect of resveratrol in a renal ischemia reperfusion *in vivo* study

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Purpose: Renal ischemia is one of the most frequent events leading to acute renal failure and it is characterized by ROS production and inflammation. Resveratrol (RSV), a wine polyphenol, protects against heart, brain and kidney ischemia. Our aim was to evaluate the nature of the nephroprotective action of RSV in a rat model of Ischemia and Reperfusion (IR).

Methods: Thirty six Wistar females rats were randomized in 3 groups: IR, IR treated with RSV 1 h before Ischemia (RSV + IR) and RSV RSV was administered i.v. (2.3 µg/Kg); animals were reperused for 5 min and 24 h and sacrificed.

Plasma, urine and renal tissue samples were collected to evaluate necrotic (Hematoxylin-Eosin) and apoptotic events (TUNEL), functional parameters (proteinuria, plasma creatinine), vitamin E content, SOD activity, COX-2 expression and the activation of the protein kinase Akt.

Results: After 5 min reperfusion vit E content and SOD activity were reduced in both IR and RSV + IR groups. After 24 h of reperfusion, RSV + IR showed tissue protection, reduced proteinuria and plasma creatinine and reduced COX-2 expression in comparison with IR. After 5 min Akt was activated in IR only ($3.96 \pm 1,242$ arbitrary units, a.u., means \pm standard error) in respect to controls (1.00 ± 0.196), while after 24 h Akt was activated in IR (3.99 ± 1.977) and RSV ($3.71 \pm 0,748$) groups but not in RSV + IR (1.31 ± 0.179).

Conclusions: In our experimental model of IR, low RSV concentration exerts nephroprotection by modulating functional parameters rather than by an anti-radical action.

Keywords: Resveratrol, kidney ischemia-reperfusion damage

Neuroprotective, hormetic effects of quercetin towards oxygen/glucose deprivation and reperfusion damages

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Background and purpose: Quercetin is one of the most widely distributed flavonoids in the plant kingdom and it is present in red wine, fruits and vegetables used in the human diet. In spite of its antioxidant and anti-inflammatory properties, its neuroprotective effects towards oxidative stress are complex and not fully understood (1). The present study has investigated the effects of quercetin (0.1–300 µM) on oxygen-glucose deprivation and reperfusion (OGD/R)-induced damages of nervous cells.

Methods and results: Rat brain slices and human neuroblastoma cells lines (IMR-32) were used. Tissues damage was assessed in brain slices after 30 min of OGD, followed by 90 min reperfusion, by measuring the release of glutamate and lactate dehydrogenase, tissue lipid peroxidation, total GSH content and water gain at the end of reperfusion phase (2). IMR-32 cells damage was assessed by measuring viability, apoptosis or necrosis 16 h after physiological bathing, subsequent to a 24 h of OGD. Results demonstrate that quercetin in both models exerted marked neuroprotective effects according to “U-shaped”, hormetic-like, concentration-response curves, with an efficacy window of 10–30 µM (brain slices) or 0.1–10 µM (IMR-32).

Conclusions: Quercetin exerts *in vitro* neuroprotective effects towards experimental oxidative stress. It remains to be seen whether the same benefit also applies *in vivo* towards brain damage induced by ischemia-reperfusion injury.

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Red wine neuroprotection in a mouse model of fetal alcohol spectrum disorders (FASD)

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Purpose: Ethanol exposure during pregnancy is a cause of mental retardation in children, inducing fetal alcohol spectrum disorders (FASD). Neurotrophic factors, such as nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF), may have a major role in FASD onset (1,2). The aim of this study was to investigate brain NGF and BDNF levels in a FASD CD-1 mouse model, following chronic early exposure to ethanol/water (11% vol) or to red wine at the same alcohol concentration.

Methods and results: No difference between groups in pregnancy duration or pups delivery, mortality and sex ratio were observed. Early ethanol exposure in adult animals disrupted the levels of both NGF and BDNF in the hippocampus and other brain areas, impaired ChAT immunopositivity in the *septum* and *nucleus basalis* and altered cognition and emotional behavior. Red wine elicited no change in behavior or in ChAT immunopositivity, but mild alterations in hippocampal BDNF and cortical NGF were observed. NGF-induced neuritic outgrowth in PC-12 cells was still present when mice were exposed to red wine, but not when exposed to ethanol/water with the same alcohol content.

Conclusions: Red wine components may exert a neuroprotective effect against ethanol-induced neurotoxicity.

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Keywords: red wine, NGF, BDNF, ethanol, mice.

Serum total antioxidant capacity level in humans after ingestion of white wine (produced following the ancient technology of Tuscany) compared to red wine

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Background and purpose: Many researchers emphasize the beneficial effects of red respect to white wine (1). Results by Girotti et al. (2) on total antioxidant capacity (TAC) of wine show that TAC of white wine decreases during the winemaking procedure.

The aim of our study was to evaluate in humans whether the ingestion of white wine, produced following the ancient technology used in Tuscany, induces an increase of serum TAC comparable to that of red wine.

Methods and results: Five male volunteers received in two subsequent sessions a fixed amount (300 mL) of white and red wine (Chianti). Blood samples were collected at time zero and after 1 and 2 h. TAC determination was performed in serum by a chemiluminescent assay (3). Following white wine ingestion, serum TAC showed a significant increase after 1 h ($p < 0.05$) as well as after 2 h ($p < 0.01$). A similar effect on TAC was observed after ingestion of red wine.

Conclusions: Serum TAC levels following white wine consumption were comparable to those measured after red wine, but higher than previously reported by our group (3), a finding possibly due to

differences in the winemaking procedure currently adopted which is different from previous ones based upon the French method.

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Keywords: White wine, red wine, anti-oxidant capacity

Are wines fined with milk or egg proteins safe for patients suffering from food allergy?

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Background and aims: Wine fining is the process of removing undesirable substances (such as polymerized tannins, phenols and proteins) by using a wide variety of substances, having both organic and inorganic nature. Egg white proteins and caseinates have useful oenological properties, but any residues left in the wines could constitute a risk for subjects suffering from food allergy. Even though quite rare in adults, milk and egg allergies can be severe and dangerous pathologies. According to the EU Directive on labeling (2003/89/EU), ingredients listed in Annex IIIa to Directive 2000/13/EC (and following amendments), and still present in wine, must be indicated in the label. The aim of this study was to investigate the possible presence of allergenic residues in 205 fined commercial wines.

Methods and results: Allergen detection and quantification were performed by immunoenzymatic and immunoelectrophoretic methods (using antibodies developed versus caseinates or egg white proteins). All samples analyzed proved to be free from detectable allergenic residues by both immunochemical tests, irrespective of the physicochemical characteristics of the wine, the type and dosage of fining agent and the oenological process used.

Conclusions: Commercial wines fined with milk or egg proteins, when treated with suitable processes, are safe for allergic patients.

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Keywords: egg allergy, wine

Beneficial effects of the Mediterranean Diet

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Purpose: The aim of this study was to evaluate the nutritional status and the quality of diet of volunteers following a Mediterranean dietary pattern [1].

Methods: A sample of 778 healthy volunteers (344 men and 434 women) underwent a full clinical examination and anthropometric measurements and completed a lifestyle and a food frequency questionnaire. On a sub-sample of 147 volunteers (71 men and 76 women), food intake was monitored by a 4-d food record and blood lipid profile and biomarkers of both redox and inflammatory status were measured.

Results: About 30% of the volunteers had a secondary school educational level; 23% of men and 31% of women had a low socio-economic level. A good adherence to the Mediterranean diet, as found in 74% of the volunteers, was associated with a lower prevalence of overweight and obesity, higher level of physical activity, reduced cardiovascular risk, oxidative stress and inflammation status; 82% of men and 61% of women consumed alcoholic beverages and, as expected for this population, alcohol consumption was mainly represented by wine (55%) and beer (47%).

Conclusions: The adherence to the Mediterranean diet, a valid model of sustainability in terms of environmental friendliness and economics, could provide a dietary option to maintain better health.

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Keywords: mediterranean diet, quality of diet

Mediterranean diet in a time of crisis: lower income, lower adherence

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Background: The Mediterranean Diet protects against cardiovascular disease and some cancers. Increasing prices of healthy food might have led Italian people to give up adherence to the traditional Mediterranean dietary pattern.

Purpose: To evaluate possible differences in eating patterns among adult Italians with different socio-economic status, with particular focus on low-income subjects.

Methods: Data from the “Moli-sani” project cohort on 24,318 citizens of the Molise region, Italy, were analysed. Two scores were used to evaluate adherence to Mediterranean diet: factor score, Prudent diet (PD) and Western diet (WD) by Principal Factor Analysis (1) of 45 food groups, from the EPIC food frequency questionnaire and the Mediterranean diet score (MDS), elaborated by Trichopoulou et al. (2). Socio-economic status was expressed as a score based on 6 variables (education, housing, ratio of live-in partners and room number (present and at childhood), availability of hot water in the home).

Results: Socio-economic status was inversely associated with adherence to both PD ($p < 0.0001$) and MDS ($p < 0.0001$) while it was positively associated to WD ($p = 0.0048$). In particular, subjects in the lower income categories showed a poor adherence to both Mediterranean eating patterns (PD < 0.0001 , MD < 0.0001) but greater adherence to a Western style diet ($p < 0.0001$). All statistical analyses were adjusted for potential confounding factors including age, sex and caloric intake.

Conclusions: The adherence to Mediterranean diet-like patterns is strongly associated with socio-economic status, in particular lower adherence with lower-income. Socio-economic status and in particular income currently represent a crucial issue for dietary choices of Italian people.

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Keywords: mediterranean diet, socio-economic status, income

The Mediterranean Diet in 2011

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Purpose: The appropriate dietary strategy to prevent chronic degenerative diseases remains a challenging and highly relevant issue. Studies in support of Mediterranean diet as an optimal diet for prevention of cardiovascular and major chronic diseases evolved rapidly in recent years. The aim of this review is to examine the available evidence of the effectiveness of Mediterranean diet in terms of health outcomes.

Methods: We conducted an updated electronic literature search through all major electronic databases.

Results: The updated review process evidenced 7 prospective studies published in the last 2 years and not included in our previously published meta-analysis (1) (1 for overall mortality, 3 for cardiovascular incidence and/or mortality, 1 for cancer incidence and/or mortality, and 2 for neurodegenerative diseases). Of note, these recent

studies included 2 health outcomes not previously investigated (i.e. mild cognitive impairment and stroke). The meta-analysis for all the studies under a random-effects model, conducted after the inclusion of these recent studies, showed that a 2-point increase of adherence to Mediterranean diet remained to be associated with a significant reduction of overall mortality (R.R.: 0.92, 95% CI: 0.90–0.94), cardiovascular incidence and/or mortality (R.R.: 0.90, 95% CI: 0.87–0.93), cancer incidence and/or mortality (R.R.: 0.94, 95% CI: 0.92–0.96) as well as occurrence of neurodegenerative diseases (R.R.: 0.87, 95% CI: 0.81–0.94).

Conclusions: This updated meta-analysis confirms, in quite a large number of subjects and studies, a significant and consistent protection

of the adherence to Mediterranean diet versus the occurrence of major outcomes associated with chronic degenerative diseases (-8% for overall mortality; -10% for cardiovascular incidence and/or mortality; -6% for cancer incidence and/or mortality and -13% for neurodegenerative diseases).

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Keywords: Mediterranean diet, chronic diseases, diet