ABSTRACTS OF THE SPEAKERS' PRESENTATIONS

1. Epidemiological Studies

OVERVIEW OF ALCOHOL AND CARDIOVASCULAR DISEASES

Klatsky AL. Senior Consultant in Cardiology Kaiser Permanente Medical Care Program, Oakland, California

The relations of alcoholic beverage consumption to various cardiovascular conditions can be approached by considering three sets of disparities. First and most basic is the difference between the harmful effects of heavier drinking and the beneficial effects of light - moderate drinking. Next are the disparities in relations between alcohol consumption and various cardiovascular conditions, making it is best to consider separately several disorders. Finally there is the area of beverage type differences, which includes possible effects of non-alcohol ingredients in the beverages. Heavy drinking, defined as usual daily consumption of three standard drinks or more, carries these increased risks: 1) Alcoholic cardiomyopathy, related only to very heavy sustained drinking in susceptible persons. 2) Systemic hypertension, an association confirmed by consistent epidemiologic data and clinical experiments, but without an established mechanism. 3) Paroxysmal supraventricular rhythm disturbances in binge drinkers (the "holiday heart syndrome"). 4) Hemorrhagic stroke, probably both subarachnoid and intracerebral. Light – moderate drinking is probably not related to increased risk of any cardiovascular condition and is consistently related to lower risk of coronary heart disease and ischemic stroke. A protective hypothesis is solidly supported by evidence for plausible biological mechanisms. Since coronary heart disease is the commonest type of heart disease there is an impact on total mortality statistics, so that light-moderate drinkers are at lower risk of death. International comparisons and some prospective studies suggest that wine is more protective against coronary heart disease than liquor or beer. Reports of possible non-alcohol beneficial components in wine (especially red) support the hypothesis of extra protection by wine, but a healthier pattern of drinking or more favorable risk traits in wine drinkers may also be involved.

MODERATE DRINKING AND CANCER

Ellison RC. Boston University School of Medicine, Boston, Massachusetts,USA

There are problems in studying the relation between moderate drinking and cancer. It is difficult to assess alcohol intake and, especially for cancer, the relevant time period of exposure may be uncertain. Further, it is becoming increasingly clear that the effects of alcohol on the risk of cancer are modified by genes, diet, and other factors. While animal models are widely used, it is not certain how they relate to cancer in humans. There are a number of cancers that are referred to as alcohol-related. These are cancers of the mouth, pharynx, larynx, esophagus, and sometimes stomach and liver. They are associated with very heavy drinking, with alcohol abuse; generally, they relate as much to heavy smoking as to heavy drinking, and do not occur with increased frequency among light-to-moderate drinkers. As for other types of cancer, there is some evidence that heavy (but not light) drinking may slightly increase the risk of lung cancer, although inadequate adjustment for confounding by smoking is a more likely cause of the associations seen. For colo-rectal cancers, recent studies suggest a slight increase in risk among men with heavy spirits consumption (but not for wine or beer, and not for moderate drinking of any beverage). Cancer of the bladder, uterus, ovary, and prostate are generally unrelated to alcohol, although some studies show a slight increase for moderate spirits drinkers for prostate cancer. For colo-rectal and bladder cancer, there is preliminary evidence that instances of increased risk among moderate drinkers occur only among people with specific genetic polymorphisms. The only common cancer often reported to be related to *moderate* alcohol consumption is breast cancer in women, and studies have shown a slight increase (about 10 %) with consumption as low as one drink per day. My colleagues and I reviewed this relation among the approximately 5,000 women in the Framingham Study, with almost 300 cases of breast cancer occurring during 25-50 years of follow up. There was no evidence of an increase in risk among women consuming any level of alcohol in that study. Since our findings were not concordant with many other studies, we also carried out a meta-analysis (41,477 incident cases from 42 studies) on the topic to see what factors might relate to different results among studies. For case-control studies, we found slightly higher estimates of alcohol effect for hospital-based rather than population-based studies; for cohort studies, longer duration of follow up was associated with lower risk. We did not find from the study that consumption of wine was different from consumption of beer or spirits in terms of cancer risk. A reduced risk of cancer has frequently been reported among alcohol drinkers for Non-Hodgkin's lymphoma, with risks that are 60-70 % lower than in abstainers in some studies. Limited recent research also suggests lower risk among moderate drinkers for thyroid cancer and, among women, for kidney cancer. For cancer mortality, large epidemiologic studies do not suggest an effect of moderate drinking on cancers other than breast cancer, and here the effect is small. While many experiments have shown that wine polyphenols, or wine itself, have strong anti-cancer effects in the laboratory, there are still only limited epidemiologic data supporting a specific protective effect of wine against cancer. A particularly interesting finding in recent research has been the identification of factors that modify the relation of alcohol to cancer. Polymorphisms of the alcohol dehydrogenase gene (ADH3) and other genes may modify the risk of a variety of cancers. Further, low intake or low serum levels of folate and other vitamins have been shown to increase the risk of cancer from alcohol consumption. We conclude (1) with the possible exception of breast cancer, moderate drinkers are not at increased risk of cancer; (2) laboratory studies suggest that wine and/or its polyphenols may have a role in protection against cancer; and (3) research in the future should focus on genetic, dietary, and other factors that modify the relation of alcohol to cancer.

ALCOHOL, WINE, DEMENTIA AND STROKE

Letenneur L. INSERM U330, Bordeaux Cedex, France

The term dementia refers to a clinical syndrome of acquired intellectual disturbances produces by brain dysfunction. Dementia may result from a wide variety of disorders, including degenerative (e.g. Alzheimer's disease, AD), vascular (e.g. multi-infarct dementia), and traumatic (e.g. head injury). Long-term abuse of alcohol is related to the development of the Wernicke-Korsakoff's syndrome or alcohol dementia. However, light to moderate alcohol intake might also reduce the risk of dementia and AD. In Bordeaux (France), a population-based prospective study

found that subjects drinking 3 to 4 standard glasses of wine per day (> 250 and up to 500 ml), categorized as moderate drinkers, the crude odds ratio (OR) was 0.18 for incident dementia ($p < 0.01$) and 0.25 for Alzheimer's disease ($p < 0.03$), as compared to the non-drinkers. After adjusting for age, sex, education, occupation, baseline cognitive performances and other possible confounders, the ORs were respectively 0.19 ($p < 0.01$) and 0.28 ($p < 0.05$). In the 922 mild drinkers (< 1 to 2 glasses per day) there was a negative association only with AD, after adjustment (OR = 0.55; $p < 0.05$). The inverse relationship between moderate wine drinking and incident dementia was explained neither by known predictors of dementia nor by medical, psychological or socio-familial factors. These results were confirmed from data of the Rotterdam study. Light-to-moderate drinking (one to three drinks per day) was significantly associated with a lower risk of any dementia (hazard ratio 0.58 [95 % CI 0.38-0.90]) and vascular dementia (hazard ratio 0.29 [0.09-0.93]). No evidence that the relation between alcohol and dementia varied by type of alcoholic beverage was found. Stroke constitutes one of the most common causes of serious functional impairment in developed countries. Ischaemic strokes represent about 80 % of all strokes. Several studies have been published and the overall conclusion is that heavy drinking is a risk factor for most stroke subtypes. Regular light to moderate drinking seemed to be associated with a decreased risk for ischaemic stroke.

ALCOHOL, BEVERAGE CHOICE AND CORONARY DISEASE

Grønbæk M. Centre for Alcohol Research, National Institute of Public Health, Copenhagen, Denmark

A large number of studies from many different countries have shown a J- shaped relation between alcohol intake and all-cause mortality. The descending leg of the curve is due to a decreased risk of cardiovascular disease among those who have a light to moderate alcohol intake. Persons with a high alcohol intake have a higher level of high density lipoprotein, which has been found to be a mediator of 40-60 % of the effect of alcohol on coronary heart disease. Correlational studies have showed that mortality from coronary heart disease is lower in countries where wine is the predominant type of alcohol, than in countries where beer or spirits are the beverages mainly ingested. Recent prospective studies from United Kingdom, Sweden and Denmark have supported the above by showing that wine drinkers are at lower mortality than beer and spirits drinkers. Several of the components of wine which may have antioxidant proporties are also present in fruits and vegetables. Therefore, diet may play a role in the interpretation of the complex relation between alcoholic beverage type and coronary heart disease mortality. In the Danish Diet Cancer and Health Study, preference of wine was associated with a higher intake of fruit, fish, vegetables, salad and a higher frequency of use of olive oil for cooking compared with preference of beer or spirits in both men and women.
In conclusion, the findings that wine drinkers are at a decreased risk of death from coronary heart disease than non-wine drinkers, suggest that substances present in wine are responsible for a beneficial effect on the outcome, in addition to that from a light intake of ethanol. However, several potential confounders remain to be excluded.

A META-ANALYSIS OF WINE, BEER AND VASCULAR RISK

Donati MB[1], Di Castelnuovo A[2], Rotondo S[2], Iacoviello L[2] and de Gaetano G[1]. [1]Center for High Technology Research and Education in Biomedical Sciences, Catholic University, 86100 Campobasso, and [2]Istituto di Ricerche Farmacologiche Mario Negri, Consorzio Mario Negri Sud, 66030 Santa Maria Imbaro, Italy

Men and women who drink an average of 1 to 2 drinks of alcohol per day have lower total mortality rates, reflected in lower incidence of coronary heart disease, diabetes, and in some populations, ischemic stroke. The clear benefit of moderate alcohol consumption on risk of coronary disease has been documented in almost 100 studies. With the general acceptance of the benefits of moderate alcohol consumption and the documented hazards of heavy consumption, research priorities of epidemiological studies have shifted to the study of possible differential health effects of beverage choice and drinking patterns. We have recently provided a quantitative summary of population studies that have reported for wine and beer the beverage-specific risk estimates for cardiovascular disease. Evidence obtained from this meta-analysis indicates an average significant reduction of 32 % of overall vascular risk associated with drinking wine. Not only were non-fatal vascular endpoints significantly reduced in wine drinkers, but also cardiovascular mortality. In studies with only males, the protection offered by wine was surprisingly small (13 %) and not significant, in contrast with studies enrolling both genders (47 %). Whether women are more susceptible to the benefit of wine or they more likely drink lower amounts taking its maximal advantage remains to be established. In relation to the reported association between moderate alcohol consumption and increased risk of breast cancer, our findings suggest that there is an overall effect of moderate wine intake on women's health. Subgroup analysis of six studies that only included people aged < 65 years showed a lower, non significant relative risk (RR) reduction by wine consumption as compared to six studies including all ages. In agreement with the relation between alcohol intake and all-cause mortality previously reported, we observed a J-shaped relationship between wine intake and vascular risk, suggesting that light to moderate wine drinkers (up to 200 ml/day on average) have lower vascular risk than either heavier drinkers or non-drinkers. Beer drinking was also found to be associated with a reduced risk of vascular events, though at an extent lower than that observed with wine (22 %). A significant inverse association was still apparent when CHD was only considered, but -at variance with wine- it did not reach statistical significance when CVD events or cardiovascular mortality were separately evaluated, likely due to the small number of available studies. Risk reduction connected with beer drinking was smaller but, at variance with wine, still significant in studies where only males were included. This suggests that women might be particularly "responsive" to alcohol itself rather than to non-alcoholic components of these beverages. Similarly to wine, beer consumption too was not associated with a significant RR reduction when six studies including only people aged < 65 years were analysed. The most important difference between wine and beer consumption was observed in the meta-analysis of studies reporting trend analysis. In contrast with wine, indeed, the fitted models failed to show any significant relationship between different amounts of beer intake and vascular risk, even when different subgroups were analyzed. Thus, the inverse association between beer consumption and vascular risk should be interpreted with

caution. When we excluded the 3 studies that did not simultaneously adjust for different types of alcoholic beverages (the most unbiased method to control for confounding), there was no difference in the RR of cardiovascular disease between wine drinkers (25 %) and beer drinkers (23 %) compared with abstainers. However, the relative 10 % overall difference between RR of wine versus beer drinkers was unchanged in studies that assessed either wine or beer drinking versus the same reference groups. The potential confounding effect of combined drinking of different types of alcoholic beverages in the same population was excluded by pooling data from studies that had taken this issue into consideration. Errors in reporting beer intake might have contributed to our failure to draw any statistically significant "dose-response" curve from studies on this beverage. Irregular (binge) drinkers possibly frequent in cohorts of beer drinkers, might have obscured a possible dose-dependent risk reduction in regular beer drinkers. In conclusion, the presence of an inverse "dose-response" relation between wine consumption and vascular risk, although of a complex, J-shaped type, is of importance. As large long-term intervention trials appear to be unfeasible for several reasons, including ethical concern, the evidence for the benefit connected with wine or beer consumption should critically include molecular and cell biology, animal and observational epidemiological studies and their meta-analysis.

ALCOHOL AND ALL CAUSE MORTALITY

Renaud S. INSERM, Unit 330, Université Bordeaux 2 – France, René Guéguen, Centre de Médecine Préventive, Nancy - France

A large number of prospective studies have observed an inverse relationship between a moderate intake of alcohol and coronary heart disease morbidity and mortality. Concerning death from all-causes, results are not unanimous. Alcohol intake was associated with a protection of all-cause mortality in England and USA physicians and the large study of the American Cancer Society. None of these studies separated the effects of different alcoholic beverages.
In our prospective studies in France on 35000 middle-aged men, we observed that only wine at moderate intake, was associated with a protective effect on all-cause mortality. The reason was that in addition to the known effect on cardiovascular diseases, a very moderate intake of wine, protected also from cancer and other causes as confirmed by Gronbaek in Denmark. Our recent results also indicate that the protective effect of a moderate intake of wine on all-cause mortality is observed at all levels of blood pressure and serum cholesterol.

2. Oxidative Stress, Antioxidants and Free Radicals

WINE PHENOLICS: STRUCTURE AND BIOLOGICAL ACTIVITIES

Waterhouse AL. Department of Viticulture & Enology, University of California, Davis

Wine is a source of diverse phenolic substances. Grapes include 5 major classes, including four classes of flavonoids. Each different class of substances has some unique activities, and within each class there are some differences between class members. In addition, phenols are actively metabolized in vivo and new studies are analyzing the effects of the metabolites, as these are the major circulating forms. These phenols have been shown to have a very wide range of biological activities from simple antioxidant effects to specific effects on enzyme systems that control iNOS expression. The observed mechanisms of action are strengthening the case that wine and other foods rich in phenols can in fact reduce chronic diseases such as heart disease and perhaps even cancer.

OXIDATIVE STRESS IN INFLAMMATORY AND FIBROTIC DISEASES

Poli G. Department of Clinical and Biological Sciences, University of Turin, Italy

An intracellular condition of biochemical imbalance between oxidative and reductive reactions towards oxidation, i.e. oxidative stress, appears to be a very common feature in chronic inflammation and fibrosis. Membrane lipids are, of course, only one but still a major and consistent biological target of oxidative stress. Indeed, an increasing bulk of scientific data is supporting an important role of lipid oxidation products in the pathogenesis of fibrosis affecting a variety of tissues. Professional phagocytes and extracellular matrix (ECM) producing cells are the key-players in bringing about an excessive fibrogenesis. Their cross-talk is triggered and favoured by a series of pro-inflammatory cytokines, with a prominent role played by the transforming growth factor b1 (TGFb1). Both expression and synthesis of this inflammatory and pro-fibrogenic cytokine are mainly modulated through redox-sensitive reactions. One lipid oxidation product able to markedly up-regulate TGFb1, is 4-hydroxynonenal (HNE) which is detectable in the exudate within the site of inflammation, is chemotactic for human monocytes and induces MCP-1 release in macrophages. Also defined oxidation products still bound to cholesterol and phospholipids have been recently demonstrated as potentially involved in inflammatory and/or fibrogenic reactions. Finally, the overall class of 27 carbon atom products of cholesterol oxidation, termed oxysterols, appears of primary interest in the progression of the atherosclerotic lesions, because of its pro-apoptotic, pro-inflammatory and pro-fibrogenic effects.

3. Structure, Activity and Bioavailability of Polyphenols

PRESENT STATUS OF ANTIOXIDANT CAPACITY MEASUREMENTS

Lissi E. Chemical Science Department, Faculty of Chemical and Biological Sciences, Santiago University of Chile, Santiago, Chile

The in vitro antioxidant capacity of pure compounds and/or complex mixtures is a matter of current interest, and a variety of methodologies have been developed for its evaluation. Most of these methodologies monitor the consumption of stable radicals, measure induction times in free radical driven reactions, and/or estimate the effect of additives upon a process that can be considered proportional to the peroxyl radical steady state concentration. However, the information obtained by these experiments is frequently underrated by lack of precision regarding what is being measured. In fact, different techniques can provide information regarding either the quantity and/or the reactivity of the measured compounds. Furthermore, depending on the type of free radical generating process, the results obtained can be related to the chelating capacity of the additives and/or be influenced by pro-oxidant activities. A meaningful interpretation of the results is often precluded by lack of knowledge regarding the kinetics and mechanism of the process. In the present work we discuss these aspects in several processes frequently employed to evaluate antioxidant capacities. In particular, it will be discussed how time profiles and kinetic data obtained over a wide range of scavenger and/or substrate concentrations can help in the interpretation of the results.

ANTIOXIDANT MECHANISMS OF POLYPHENOLIC CAFFEIC ACID OLIGOMERS, CONSTITUENTS FROM *SALVIA OFFICINALIS*

Bors W[1], Michel C[1], Stettmaier K[1], Lu Y[2] and Foo LY[2]. [1]Inst. Strahlenbiol., GSF Research Center, D-85764 Neuherberg, Germany and [2]Gracefield Research Center, P.O. Box 31-310, Lower Hutt, New Zealand

Caffeic acid, rosmarinic acid and oligomers of caffeic acid with multiple catechol groups are all constituents of *Salvia officinalis*. Their antioxidant potential was investigated with regard to their radical scavenging activity and the stability and structure of the intermediate radicals. Pulse-radiolytic studies revealed very high rate constants with hydroxyl and azide radicals. Evidence from kinetic modelling calculations suggested unusual complex behavior due to the presence of both O_4- and O_3-semiquinones and formation and decay of a hydroxyl radical adduct at the vinyl side chain. The radical structures observed by EPR spectroscopy after autoxidation in slightly alkaline solutions were only partially identified due to their instability and generally represented dissociated O_4-semiquinones. Hybrid density-functional calculations of the potential radical structures showed distinct differences between the resonance stabilization of the O_4- and O_3-semiquinones of caffeic and dihydrocaffeic acids, reflected also in the considerably faster decay of the *meta* (O_3) semiquinones observed by pulse radiolysis. No evidence was found for dimerization reactions via Cb radicals typical for lignin biosynthesis.

INTERACTION BETWEEN BILITRANSLOCASE AND PHENOLICS

Mattivi F[1], Passamonti S[2] and Vrhovsek U[1]. [1]Istituto Agrario di San Michele all'Adige, Dipartimento Laboratorio Analisi e Ricerche, San Michele all'Adige, Italy. [2]Dipartimento di Biochimica Biofisica e Chimica delle Macromolecole, Università di Trieste, Trieste, Italy

Bilitranslocase (TC 2.A.65.1.1) is a plasma membrane carrier located both in the epithelial layers of the gastric mucosa and at the sinusoidal domain of the liver cells. It is involved in the transport of bilirubin and other organic anions from the blood into the liver. Moreover, it has been suggested it does play a role in nutrition, promoting the absorption of the vitamin PP precursor nicotinic acid from the food. Also, some important phenolics, and in particular the majority of the anthocyanins occurring in the human diet are powerful inhibitors in the in vitro bilitranslocase transport activity assay, with Ki values in the range of 1.4-22 μM. This lecture discusses the possible mechanisms of interaction between anthocyanins and bilitranslocase, explaining the variability of their Ki values; it presents a survey on the presence of the different structures of anthocyanins in red wine, grape and other red fruits, and provides some "in vivo" data supporting the hypothesis of a role of bilitranslocase in the absorption of the anthocyanins.

VARIABILITY IN ANTIOXIDANT COMPOUNDS IN AGROINDUSTRIAL FOODS. IMPORTANCE OF RAW MATERIAL: THE CASE OF WINE

Peña-Neira A. Department of Agroindustry and Enology, Faculty of Agronomical Sciences, University of Chile, Santiago, Chile

The concentration and presence of the different compounds of the grape berry (*Vitis vinifera L.)* depends, among other factors, on the stage of ripeness of the fruit, genetical factors (cultivar, clone, rootstock, etc) and enviromental factors (climate, microclimate, type of soil, water supply, etc.). Aproximatly the 40-60 % of the total phenolic compounds of the berry are extracted to wine. Not all the phenolic compounds have the same antioxidant and pharmacological properties, the raw material used in wine production being very relevant as in other agroindustrial products (olive oil, juices, etc). In this talk I present aspects about the evolution of some phenolic compounds during the ripening of grapes and also the differences in phenolic composition among varieties, clones, grapes and wines from different Chilean areas of production for the same variety (cv. Carmenere).

HEALTHY EATING AND DRINKING

Crozier A. Inst. of Biomedical and Life Science, University of Glasgow, United Kingdom

The antioxidant capacity of almost 100 fruits, vegetables, nuts and red wine has been screened as the identification of high antioxidant produce makes it possible to increase antioxidant intake without major changes in dietary habits. Broad beans and red and yellow peppers showed high activity, as did red fruit including plums, blueberries, strawberries and raspberries. Peanuts were also rich in antioxidants. Fractionation studies indicate that the compounds responsible for the antioxidant activity of red

fruits and berries are different from those present in broad beans and peppers. Red wines showed a range of antioxidant and vasodilation activities with highest levels being observed in wines prepared from small thick skinned grapes, such as Cabernet Sauvignon, that are grown in a reliably warm dry climate and which are left on the vine to thoroughly ripen. The antioxidant capacity of red wines greatly exceeds that of red grape juices and similarly grapes for making red wine are a much richer source of antioxidants than table grapes. Details will be presented of the LC-MS-MS analysis of phenolic compounds in raspberries and red wine and on the appearance of phenolics in the bloodstream and urine after the consumption of these products.

4. Oxidative Stress and Polyphenols

AGEING, OXIDATIVE STRESS AND FLAVONOIDS

Viña J, Pereda J, Borrás C, Lloret A, Gómez C and Sastre J. Departament of Physiology, Faculty of Medicine, Universitat de Valencia, Spain

The role of mitochondria in the generation of oxidative stress which is important to explain age-associated cellular damage was first proposed by Miquel. We showed that mitochondria are damaged inside intact hepatocytes from old animals. Furthermore, oxidation of glutathione which occurs upon ageing takes place preferentially in mitochondria. In fact, oxidation of mitochondrial glutathione may account for the oxidation of all cellular glutathione associated with ageing. Moreover, oxidative damage to mitochondrial DNA which occurs upon ageing is correlated with oxidation of mitochondrial glutathione. Work from Sohal and also from Barja has shown that the rate of oxidant production by mitochondria is critical to explain damage associated with ageing. Recently, we have become interested in determining the reason for the greater longevity of females over males. We have found that mitochondria from females produce lower levels of peroxides than those from males. Furthermore, they have higher levels of glutathione and lower levels of oxidative DNA damage than their male counterparts. The reason for this is that oestrogens induce the expression of antioxidant enzymes such as mitochondrial glutathione peroxidase and superoxide dismutase.
Treatment of animals with flavonoid mixtures such as *Ginkgo biloba* extracts partially prevents this damage. Indeed, *Ginkgo biloba* prevents oxidant production by mitochondria, damage to DNA and oxidation of glutathione. In a similar fashion, animals fed with red wine produce fewer peroxides and have a higher level of mitochondrial glutathione than controls. This is due to the non-alcoholic fraction of wine. Results showing the beneficial effects of flavonoids and also of phytoestrogens will be discussed.

WINE FLAVONOIDS AS PEROXYNITRITE SCAVENGERS

Boveris A, Álvarez S and Valdez L. Laboratory of Free Radical Biology, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina

Three main differentiated spaces for free radical reactions are recognized in the human body: the intracellular space, the vascular space, and the intestine. The general pathway of the propagation reactions is similar for the three spaces, but there are important differences in the initiation reactions and in the involved antioxidants. In the vascular space the initiation reactions are provided by the homolysis of plasma $ONOO^-$ and ROOH. Peroxynitrite is produced by the termination reaction of O_2^- and NO, that are vectorially produced towards the extracellular space by leukocytes (neutrophils, macrophages and lymphocytes) and by endothelial cells. Plasma $ONOO^-$ is able to initiate lipoperoxidation and to nitrate LDL, the atherogenic plaque and BSA. ROOH homolysis occurs in the LDL surface, catalyzed by metal-containing (Cu, Fe) plasma proteins. Flavonoids behave as effective antioxidants in preventing free radical oxidations (lipoperoxidation) and nitration; IC_{50} for some flavonoids (myricetin) are in the 20-40 mM range. Extracts of *V. vinifera* seeds show IC_{50} in the range of 60-80 mg/ml. Red wine at about 1-2 ml/l (equivalent to 1 glass/day) was found in vitro to decrease to one-half a series of oxidative model reactions. The intestine is the main source of plasma ROOH. Plasma triglycerides and LDL-bound ROOH are increased after a meal in the postprandial period. The flavonoids, taken as part of the normal diet (about 0.5 g/day) provide an antioxidant capacity in the intestine (5 mmol ROOH), which is about 5 and 10 times higher than the antioxidant capacity of vitamins C and E in the intestine. It is concluded that red wine and other flavonoid-rich foods provide antioxidants that effectively prevent postprandial oxidative stress in the vascular space.
Supported by Fondo Vitivinícola Mendoza, Mendoza, Argentina.

HOMOCYSTEINE-INDUCED OXIDATIVE STRESS IN ENDOTHELIAL CELLS AND POLYPHENOLIC ANTIOXIDANTS

Foncea R, Martínez A, Carvajal C and Leighton F. Cellular and Molecular Biology Department, Faculty of Biological Sciences, Catholic University of Chile, Santiago, Chile

Increase in plasma homocysteine (Hcy) is a risk factor of atherosclerosis and vascular dysfunction. It has been suggested that Hcy increases the reactive oxygen species (ROS) generation, but the mechanisms leading to vascular dysfunction are unclear. We studied the effect of Hcy (250-500mM) on oxidative stress induction and the role of ROS in the Hcy-induced signaling pathways, nitric oxide (NO) levels, NFkB activation, nitrotyrosine content and the expression of adhesion protein VCAM1, in human umbilical vein endothelial cells (HUVEC). Also, the protective capacity of the polyphenolic antioxidants. Stimulation with Hcy resulted in a dose-dependent increase of ROS and a correlated decrease of intracelular NO. Hcy increases tyrosine kinase activity with a phosphotyrosine stimulation of ERK5, Scr and p38. A rapid and transient activation of ERK1/2 was also detected. Additionally, incubation with Hcy resulted in the rapid activation of NFkB (30 min), an increase on nitrotyrosine content and the induction of VCAM1. These effects were inhibited by the polyphenolic antioxidants. These results support the participation of ROS in Hcy-induced endothelial dysfunction and the protective effect of polyphenolic antioxidants.
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POTENTIAL HEALTH EFFECTS OF FLAVAN-3-OLS AND PROCYANIDINS: BEYOND FREE RADICAL SCAVENGING

Fraga C, Fisicoquimica-PRALIB, School of Pharmacy and Biochemistry, University of Buenos Aires, Argentina

The regular consumption (14 days) of a flavonoid-rich milk chocolate in a group of steadily trained young (18-20 year old) males was associated with a decrease in the blood pressure (BP) and a subtle decrease in plasma malondialdehyde (MDA). These events were not statistically correlated. The consumption of the chocolate did not produce changes in BP and MDA in the 6 hours following ingestion. One mechanism that can contribute to a reduction in BP is an inhibition of ACE. To examine this concept, the ability of chocolate flavan-3-ols and related procyanidins to inhibit ACE in vitro was evaluated. It was determined that, flavanols and related procyanidins added to human plasma at micromolar concentrations, inhibited ACE activity. Furthermore, incubation of purified ACE with high chain length procyanidins (hexamers), demonstrated a significant inhibition of the enzyme by these compounds. Considering that enalapril, a specific ACE inhibitor, has several antioxidant effects and can regulate nitric oxide production, we propose a physiological connection among the renin-angiotensin system, oxidative stress status, nitric oxide production, and flavonoids. This connection could explain some of the putative effects of flavanols and procyanidins with respect to vascular health.
Supported with grants from the University of Buenos Aires (B042 and ANPCyT (PICT 2000-8951). Mars Inc., USA, provided the flavonoids-rich chocolate and purified flavanols and procyanidins used in the studies.

OXIDATIVE STRESS-INDUCED ENDOTHELIAL CELLULAR RESPONSE AND ITS INHIBITION BY RED WINE EXTRACTS

Naito Y, Manabe H, Shimozawa M, Tomatsuri N, Uchiyama K, Takagi T, Yoshida N and Yoshikawa T. First Department of Medicne, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan

Recent evidence suggests a role for the inflammatory response in the pathogenesis of atherosclerosis with the adhesion of circulating monocyte to the endothelium at the site of injury. Leukocytes adhere and emigrate into the subendothelial space in response to chemoattractants and other activating molecules, which are mediated by adhesion molecules located on leukocyte/ endothelial cells. We have reported using human aortic endothelial cells (HAEC) that the expression of ICAM-1 and VCAM-1 is upregulated by the treatment with oxidized LDL, lysophosphatidylcholine, tumor necrosis factor-alpha, and 4-hydroxynonenal, and that the increased expression is inhibited by alpha-tocopherol as well as by functional ingredients of food factors such as rosmarinic acid. In the present paper, we will review our recent data and present the inhibitory effect of red wine extracts against the endothelial inflammatory response to oxidative stress. Red wine extracts significantly inhibited the increase in the surface expression and mRNA levels of these adhesion molecules as well as the production of monocyte chemoattractant protein-1 from the HAEC in a dose-dependent manner. The treatment of HAEC with the extracts siginificantly reduced the adherence and the transmigaration of monocytes. These anti-inflammatory properties of red wine extracts suggest that red wine may have potential benefits in atherosclerosis.

5. Haemostasis, Wine and Alcohol

ALCOHOL, WINE AND HEMOSTASIS: AN OVERVIEW

de Gaetano G[1], Rotondo S[2], Rotillo D[2], Lorenzet R[2] and Cerletti C[2]. [1]Center for High Technology Research and Education in Biomedical Sciences, Catholic University, 86100 Campobasso, and [2]Istituto di Ricerche Farmacologiche Mario Negri, Consorzio Mario Negri Sud, 66030 Santa Maria Imbaro, Italy

Studies showing that alcohol favourably influences lipid profile as well as hemostatic/thrombotic factors give experimental support to the inverse association between moderate alcohol intake and cardiovascular disease. A strong and consistent evidence has been provided linking moderate alcohol intake (30g ethanol/day) with higher concentrations of high density lipoprotein (HDL) cholesterol and apolipoprotein A-I (apoA-I) and lower fibrinogen levels. The mechanisms through which wine might exert antiatherogenic and antithrombotic effects appear to be distinct from those of alcohol and, at least in part, attributable to biological properties peculiar to its polyphenolic constituents, that are recognised antioxidants and radical scavengers. Different polyphenols have been shown to modulate the function of cellular components involved in the process of hemostasis and thrombosis in several systems. Modulation of vascular response, possibly through mechanisms linked to the nitric oxide (NO) pathway, may contribute to the maintenance of blood vessel function. Interference with the arachidonic acid metabolism in both platelets and leukocytes has been reported, which resulted in inhibition of platelet aggregation and reduced synthesis of pro-thrombotic and pro-inflammatory mediators in vitro, in experimental models and in humans. Furthermore, some polyphenols can modulate specific pathways regulating the expression and activation of genes induced by a variety of agonists; this results in down-regulation of the expression of adhesive molecules and tissue factor activity in both endothelial cells and leukocytes, and ultimately in functional modulation of cell-cell interactions and procoagulant activities. We suggest that ethanol itself has potential beneficial effects on the cardiovascular system, mainly increasing HDL-cholesterol and decreasing oxidation of LDL. Wine may give additional benefits due to its greater antioxidant and anti-inflammatory effects, which result in down-regulation of the hemostatic system. At least part of the effects of red wine should be attributed to its content in polyphenols.

MECHANISMS OF ALCOHOL AND POLYPHENOL CARDIOPROTECTION

Booyse FM. Division of Cardiovascular Disease, University of Alabama at Birmingham, Birmingham, Alabama, USA

Moderate alcohol or red wine consumption reduces the risk for CHD and CHD-related mortality. This overall cardioprotection may be mediated, in part, by a combination of diverse altered vascular, cellular and biological functions, including an increase in fibrinolysis. Endothelial cell (EC)-mediated fibrinolysis is regulated through the synthesis of fibrinolytic proteins (t-PA, u-PA and PAI-1) and is localized at the EC surface via specific receptors (Rs) for PAs (PARs) and circulating plasminogen (PmgRs). EC-mediated fibrinolysis requires the complex multi-component interactions of PAs, PAI-1, PARs and Pmg:PmgRs to facilitate the activation of EC-bound Pmg at the cell surface. Consequently, if systemic factors (i.e. alcohol and/or red wine components, in particular principal polyphenols) altered the expression/interaction of one or more of these EC fibrinolytic components to increase fibrinolysis, this would be expected to decrease the risk for early fibrin deposition, atherosclerosis/CHD, as well as the atherothrombotic consequences of MI, resulting in reduced CHD and CHD-related mortality. Described studies will identify/define the molecular regulatory mechanisms by which moderate alcohol (≤ 0.1 %, v/v) and/or principal polyphenols (catechin, epicatechin, quercetin, resveratrol) affect EC PAs, PAI-1, PARs and PmgRs expression to increase fibrinolysis, in vitro, in cultured human ECs and *in vivo*, in rat/mouse aortic endothelium. In vitro studies will describe ethanol-/polyphenol-induced effects on the kinetics of increased EC-bound Glu-Pmg activation, concomitant with the up-regulation of PAs (t-PA, u-PA), PARs (annexin II, u-PAR) and PmgRs (annexin II, a-enolase) antigen, ligand binding activity, mRNA and gene transcription and simultaneous down-regulation of PAI-1 antigen, mRNA and gene transcription. Additional studies will show that ethanol and polyphenols share an early signal transduction pathway by common activation of the p38 MAPK cascade, resulting in the coordinate gene transcription of t-PA, u-PA, u-PAR and annexin II. In vivo studies will describe effects of acute (<24 hr) and chronic (>2 wk) ethanol or polyphenol(s) treatment on t-PA, u-PA and PAI-1 antigen and mRNA expression in rat/mouse aortic endothelium. Studies to evaluate the effects of chronic ethanol-/polyphenol-induced increased fibrinolysis, in vivo, on clot lysis in a C57/B-6 mouse model of thrombosis, will show a significant increase in the rate of spontaneous thrombolysis. In concert, results gleaned from these in vitro and in vivo studies will increase our understanding of the molecular mechanism(s) by which ethanol and/or polyphenols up-regulate EC fibrinolysis and will provide a well-defined molecular basis by which increased fibrinolysis can contribute to the overall cardiovascular disease protective mechanisms attributed to moderate alcohol or red wine consumption.

HOW COULD WINE CONSUMPTION BE PROTECTIVE AGAINST CARDIOVASCULAR EVENTS?

Drouet L, Philippe C, Soulat T, Bal dit Sollier C and Rabot S. Département d'Angio-Hématologie Hopital Lariboisière Paris, UEPSD INRA Jouy-en-Josas, France

Cardiovascular events are the first cause of mortality and morbidity in industrialized countries. The lower than expected incidence of cardiovascular events in French population is known as the French Paradox. This paradox is emphasized by the high incidence of regular risk factors (mainly high cholesterol and lipid intake) in the French population. Clinical cardiovascular events are mainly of atherothrombotic origin. "Atherothrombosis" associates two fundamentals:
-Atherosclerosis, which are the lesions affecting the vessel wall of arteries of medium diameter of the entire arterial tree.
The reactive thrombosis occurring on these evolved lesions specially when they rupture. Without a thrombotic reaction even the most evolved arterial lesions would remain clinically silent.

Atherosclerotic lesions are triggered by a genetic background associating numerous metabolic dysfunctions (hypercholesterolemia, hyperhomocysteinemia, hyperglycemia, and hypertension) together with environmental factors. Those environmental factors are mostly nutritional factors which aggravate the metabolic dysfunctions (high lipid and cholesterol intake, hyperhomocystinemia, high sugar intake (mostly rapid action sugar), high sodium intake…)) together with other types of factors participating in vascular lesions (smoking, chronic inflammation and infection…). The lesion unstability is the result of the state of development of the lesion and of triggering factors which are mostly inflammatory and infectious factors. The thrombotic reactivity depends also on the genetic background and some predisposing conditions (hypercoagulability, hyperviscosity etc.…). As the emphasis on the pathophysiogeny of atherosclerosis has traditionally focused on lipid abnormalities, most of the literature on the potential beneficial effect of regular and moderate wine consumption has studied the effect of this diet specificity on lipid parameters.We wanted to study the effect of wine consumption on the global disease, both on the arterial lesions and on the thrombotic reactivity. As far as the thrombotic reactivity several data from the literature have given argument for a potential benefit of regular and moderate wine intake on: platelet function, fibrinogen level, leucocyte procoagulant activity etc. We have gathered some personal epidemiological data mainly with the French Toulouse Monica cohort (collaboration with J Ferrieres, JB Ruidavets et P Marques-Vidal) showing that on general population the potential beneficial effect of regular and moderate wine consumption on coagulation and vascular parameters is hidden by the concomitant high tobacco consumption in most of the wine drinkers.

To allow a more simplified approach we used an animal model. For the study of atherosclerotic lesions and thrombotic reactivity we used a strain of mouse whose gene for Apo E lipoprotein has been knocked out. This genetic manipulation triggers spontaneous hypercholesterolemia and early development of aortic lesions (3 months). To quantify the thrombotic reactivity, a chamber of *ex vivo* thrombogenesis specially sized to the mouse was developed. Four groups of 24 animals each were studied in parallel: - Control group (receiving only acidified water for drinking (pH 3.3)), - alcohol group (receiving 10 % alcohol in drinking water(pH3.3)), - catechine + alcohol group (160mg/L pure catechine + 10 % alcohol in drinking water (pH3.3)), - wine extract + alcohol group (3g/L red wine extract + 10 % alcohol in drinking water (pH3.3)). The alcohol and phenol (wine extract or pure cathechine) consumption was calculated from a regular human wine consumption. Animals four weeks old were treated for 3 months and blood parameters, thrombotic reactivity and atherosclerotic lesions were quantified. Interestingly and quite unexpectedly: red wine extract and catechine not only did not prevent atherosclerotic lesions but if a trend was noticed it was in favor of a stimulation of the atherosclerotic lesion in these groups. As this effect was also noticed in the alcohol group that could be an alcohol effect which could hide an other potentially beneficial effect of wine extract or catechine absorption. These results are in keeping with the results recently published by Bentzon et al. (Circulation 2001). But additionally in the same groups of animals while no beneficial effect on atherosclerosis was noticed, a highly significant reduction of the thrombotic reactivity was observed in the wine extract group, to a significant but less limited extent in the catechine group. Alcohol had no significant effect on this thrombotic reaction.

Taken all together these results sustain the hypothesis that if regular and moderate absorption of wine reduces the incidence of ischemic cardiovascular events, this protection is not on the extent of the arterial lesions but on the thrombotic reactivity.

DIFFERENTIAL EFFECTS OF RED WINE AND DIET ON HEMOSTASIS.

Mezzano D. Department of Hematology-Oncology, School of Medicine, Catholic University of Chile, Santiago, Chile

We compared the effect of alcohol-free Mediterranean-type diet (MD) and high-fat diet (HFD) on plasma concentration of emergent haemostatic cardiovascular risk factors (HCVRF) and on variables of primary haemostasis (bleeding time, plasma von Willebrand factor and platelet aggregation/ secretion). Moreover, we tested if red wine supplementation of both diets modifies these variables, independent of diet. In a controlled prospective intervention study, two groups, each of 21 healthy male university students (22 ± 3.4 years), received either MD or HFD during 90 days. Between days 30-60, both diets were supplemented with 240 ml/day of red wine. Baseline (T0) and T30, T60 and T90-day samples were drawn. Bleeding time was measured before (day 30) and after (day 60) wine supplementation. No drop out from the study was observed. Results. As expected, on day 30, individuals on MD had significantly higher levels of plasma beta-carotene, folate, ascorbate, and eicosapentaenoic acid in plasma lipid fractions, than those on HFD. Total plasma cholesterol, HDL and LDL did not change significantly in either study group at any time point. Volunteers on HFD at T30 had increases in pro-coagulants fibrinogen (22 %), factor VIIc (9 %), and factor VIIIc (4 %), and decreases in natural anticoagulants antithrombin III (3 %), protein C (11 %) and protein S (6 %) and of 20 % in plasminogen activator inhibitor-1. At the same time, individuals on MD had marginal increases in fibrinogen (4 %), antithrombin III (5 %), protein C (3 %), protein S (2.7 %), and decreases in factor VIIIc (9 %), and plasminogen activator inhibitor-1 (21 %). After adjusting by baseline values, MD was associated with lower plasma fibrinogen ($p = 0.03$), factor VIIc ($p = 0.034$) and factor VIIIc ($p = 0.0057$) and with higher levels of protein S ($p = 0.013$). Red wine supplementation, in both diets, resulted in decreased plasma fibrinogen ($p = 0.001$) and factor VIIc ($p = 0.05$), and increased tissue plasminogen activator antigen ($p = 0.01$) and plasminogen activator inhibitor-1 antigen ($p = 0.0003$). Wine consumption was also associated with significantly ($p = 0.01$) divergent effects on antithrombin III: it decreased by 10 % in individuals on HFD but increased slightly in those on MD. With regard to primary haemostasis variables, individuals on MD had longer bleeding time (BT) than those on HFD (7.6 ±2.8 vs. 5.8 ±1.7 min; $p = 0.017$) measured at T30. BT did not change significantly after 1 month of wine supplementation (7.1 ±2.0 vs. 5.5 ±2.0 min, respectively). Plasma concentration of von Willebrand factor (vWF:Ag) was not significantly different between diet groups at baseline and did not change significantly at 30, 60 or 90 days. MD intake was associated with an increase in platelet serotonin secretion ($p = 0.02$) after stimulation with epinephrine ($p = 0.07$). Wine intake resulted in a marginal decrease in platelet ^{14}C-5-HT secretion with 4 mM ADP ($p = 0.07$), whereas both platelet aggregation and secretion were consistently increased when using collagen as agonist ($p \leq 0.01$). No effects of diet or wine were detected in plasma protein C and C-reactive protein.

Conclusion: MD and moderate consumption of red wine have complementary, mostly beneficial effects on haemostatic CV risk factors. The longer BT in individuals on MD, independently of red wine, denotes less interaction of platelets with the vascular wall, which could be beneficial from the point of view of cardiovascular (CV) risk. This effect is not explained by changes in the measured haemostatic determinants of BT (plasma vWF, ex vivo platelet function), and might be attributed to other as yet unknown vascular factors. Unexpectedly, moderate consumption of red wine results in a significant increase in ex vivo platelet aggregation and secretion after stimulation with collagen. This observation contradicts previous reports, though further studies are required to elucidate the influence of this finding in CV risk.

ALCOHOL, WINE AND PLATELET FUNCTION

Ruf JC. Enology - Wine, Nutrition and Health - Methods of Analysis Unit, International Wine and Vine Office, Paris, France

Epidemiological studies have demonstrated an inverse correlation between moderate wine and alcohol consumption and morbidity and mortality from coronary heart disease (CHD). This protective effect has been associated with an increase in the plasma level of high density lipoprotein (HDL)-cholesterol, as it is well known that plasma HDL is inversely correlated with CHD. In addition, it has become evident that blood platelets contribute to the rate of development of atherosclerosis and CHD through several mechanisms. Recent studies have shown HDL-cholesterol levels can explain only 50 % of the protective effect of alcoholic beverages. The other 50 % may be partly related to decreased platelet activity. The antiplatelet activity of wine is explained not only by ethanol but also by the polyphenolic components with which red wines are richly endowed. Several studies carried out in humans and animals have shown that wine phenolics could exert their effects by reducing prostanoid synthesis from arachidonate. In addition, it has been suggested that wine phenolics could reduce platelet activity mediated by nitric oxide. Moreover, wine phenolics increase vitamin E levels while decreasing the oxidation of platelets submitted to oxidative stress. However, a rebound phenomenon of hyperaggregability is observed after acute alcohol consumption but not after wine consumption. This protection afforded by wine has been duplicated in animals with grape phenolics added to alcohol. This rebound phenomenon could explain ischemic strokes or sudden deaths known to occur after episodes of drunkenness. It appears that wine and wine phenolics in particular could significantly inhibit platelet aggregation and that this could explain, at least in part, the protective effect of red wine against atherosclerosis and coronary heart disease.

6. Plasma Lipids

WINE FLAVONOID ANTIOXIDANTS AGAINST LDL OXIDATION AND CARDIOVASCULAR DISEASES

Aviram M. Lipid Research Laboratory, Technion Faculty of Medicine, The Rappaport Family Institute for Research in the Medical Sciences and Rambam Medical Center, Haifa, Israel

Oxidation of low density lipoprotein (LDL) is a major risk factor for atherosclerosis and hence search for potent natural antioxidants, such as wine flavonoids, against LDL oxidation is the focus of extensive research. Red wine, but not white wine consumption (400 ml per day, for a period of 2 weeks) by healthy volunteers resulted in a reduced propensity of the volunteers' LDL to lipid peroxidation. Processing of white wine by imposing a short period of grape skin contact in the presence of alcohol leads to the extraction of skin's polyphenols and produces polyphenol-rich white wine with antioxidant characteristics similar to those of red wine. The climate conditions under which grapes are grown could explain the increased content of flavonols and hence, the high antioxidant potency of wine derived from grapes exposed to sunlight. We next studied the effects of wine flavonoids on oxidative stress and atherosclerosis progression by using atherosclerotic mice. The atherosclerotic lesion area in apolipoprotein E deficient (E°) mice was decreased by 45 % in mice treated with red wine or its major flavonoids, in comparison to placebo-treated E° mice. These results were associated with a 47 % reduced susceptibility of LDL to oxidation and a 55 % decreased susceptibility of LDL to aggregation. Finally, HDL-associated paraoxonase (PON1-an esterase whose activity is associated with protection against lipid peroxidation and atherosclerosis) activity was preserved in red wine-treated mice in comparison to placebo-treated mice (where enhanced lipid peroxidation and atherosclerosis were associated with a decrement in paraoxonase activity).We thus conclude that dietary consumption of wine flavonoids by humans or by the atherosclerotic E° mice, substantially decreased their LDL oxidation and aggregation, as well as atherosclerosis progression. These effects could be related to the wine specific flavonoids (such as some specific flavonols) and to the preservation of PON1.

ROLE OF SR-BI RECEPTOR IN CELLULAR CHOLESTEROL AND TOCOPHEROL METABOLISM

Rigotti A. Gastroenterology Department, Faculty of Medicine, Catholic University of Chile, Santiago, Chile

Previous studies have indicated that selective lipid uptake from lipoproteins is important for cholesterol and a-tocopherol (α-T) delivery to cells. Here we show that the scavenger receptor class B type I (SR-BI) mediates efficient transfer of cholesteryl esters and α-T from high density lipoproteins (HDL) to cultured cells. In addition, SR-BI-deficient mice, relative to wild-type control animals, exhibit a substantial increase in plasma cholesterol and α-T levels that were due to an elevated content of these lipids in abnormally large plasma HDL particles. The lack of SR-BI in mice also impaired reverse cholesterol transport due to decreased biliary cholesterol elimination as well as decreased α-T contents of both bile and several tissues, including ovary, testis, lung and brain. These findings show

that SR-BI plays an important role in regulating plasma cholesterol and α-T metabolism and in transferring these lipids from lipoproteins to specific tissues. The abnormal metabolism of lipoprotein cholesterol and α-T in SR-BI-deficient mice may be relevant to the reproductive and cardiovascular pathologies exhibited by these animals.

WINE, POST-PRANDIAL OXIDATIVE STRESS AND FORMATION OF ATHEROGENIC LDL-

Ursini F. Department of Biological Chemistry, University of Padova, Italy

Wine, taken with food, minimizes the post-prandial rise of plasma lipid hydroperoxides and abolishes the post-prandial increase of LDL oxidability. This effect had been attributed to procyanidins. These are the most efficient antioxidants among wine polyphenols containing catechol groups due to the hydrogen transfer mechanism of the radical scavenging reaction, which renders the reaction compatible with the pH of the stomach where lipid peroxidation is sparked by lipid hydroperoxides present in the food. Thus, eating a meal containing oxidized lipids and a catalyst of peroxidation such as myoglobin, accounts for an atherogenic risk factor that is minimized when the food is taken with wine. The effector of the post-prandial atherogenic challenge is LDL-, an oxidatively modified lipoprotein, where secondary structure and conformation of apoB are deeply altered. Polar lipid oxidation products in LDL- produces an alteration of surface lipid packing, in turn leading to a loss of alpha-structure of apoB and a corresponding increase of beta-structure. This is thought to facilitate aggregation of lipoproteins, which become cytotoxic and resistant to proteolysis. In this respect, atherogenesis could be considered as a disease produced by accumulation of misfolded lipoproteins able to elicit the inflammatory response typical of the early phases of the disease. By minimizing the post-prandial increase of lipid hydroperoxides, wine prevents the formation of LDL- and this accounts for a major anti-atherogenetic effect.

7. Endothelium-dependent Vascular Reactivity

ENDOTHELIAL FUNCTION AND DIET

Cuevas A. Nutrition Department, Faculty of Medicine, Catholic University of Chile, Santiago, Chile

Endothelial dysfunction is one of the earliest events in atherosclerosis development, and is also important in established coronary artery disease where loss of endothelium-dependent relaxation may induce dynamic narrowing at lesion sites. A major consequence of endothelial damage is lower bioavailability of nitric oxide (NO), the most potent endogenous vasodilator. In addition, NO inhibits platelet aggregation, smooth muscle cell proliferation and adhesion of monocytes to endothelial cells. Endothelial dysfunction is also present in patients without clinical evidence of cardiovascular diseases but who have coronary risk factors, such as hypertension, dyslipidemia, diabetes or smoking. At present, measurement of soluble plasma markers and non-invasive techniques, mainly high resolution ultrasound of the brachial artery or other superficial arteries, has provided a simple tool for study of endothelial function and the potential effects of pharmacological and non-pharmacological interventions. Particularly, it has been demonstrated that dietary factors may induce significant changes on vascular reactivity. Several nutrients such as fish oils, antioxidant vitamins, polyphenols, L-arginine, folic acid and soy protein have showed an improvement in endothelial dependent vasodilation that can mediate their cardioprotective effects. Inversely, high fat diets (especially saturated fat) on acute or chronic administration may decrease vascular reactivity increasing the risk of cardiovascular diseases. Moreover, attention has been focused on dietary patterns in populations with lower prevalence of cardiovascular disease. There are some evidences suggesting that Mediterranean diet characterized by high consumption of vegetables, fish, olive oil as main fat, and moderate consumption of wine, may have a positive effect on endothelial function. Likewise, the diet pattern of Asian populations with high consumption of soy products, tea and fish may improve vascular reactivity. Inversely, a dietary intervention with a Western diet showed a deleterious effect on endothelial dependent vasodilation. These results give us evidence on the significant role of diet on endothelial function and its impact on the pathogenesis of atherosclerosis.

THE EFFECT OF RED WINE ON ENDOTHELIAL FUNCTION AND OXIDATIVE STRESS IN PATIENTS WITH ACUTE CORONARY SYNDROME

Guarda E[1], Godoy I[1], Foncea R[2], Pérez DD[2], Romero C[1], Venegas R[1] and Leighton F[2]. [1]Faculty of Medicine and [2]Faculty of Biological Sciences, Catholic University of Chile, Santiago, Chile

Moderate red wine consumption may improve endothelial function in normal volunteers. This can be related with antioxidant properties present in red wine. Since in coronary patients both endothelial dysfunction and oxidative damage play an important role, we sought to explore the effects of moderate red wine consumption on these parameters in patients with an acute coronary syndrome (ACS).
We enrolled 20 patients treated with PTCA and stent after an acute coronary event (unstable angina and non-Q AMI).

All patients received treatment with step 2 AHA diet, aspirin, clopidogrel, betablockers and atorvastatin. Patients were randomly selected to a red-wine group (n= 9, 250 ml daily, Cabernet Sauvignon variety) or to a control group (n= 11, abstinence from alcoholic beverages). Studies were performed at baseline and after 2 months. Endothelial function was estimated by flow mediated vasodilatation of the brachial artery using a 3-11 mHz linear array transducer at 1, 2 and 3 min after releasing the cuff pressure. To evaluate plasma antioxidant capacity, we measure total antioxidant reactivity (TAR) using a free radical generator and chemiluminiscence, and ferric reducing antioxidant power (FRAP). Oxidative damage was evaluated by 8-OH deoxyguanosine measurements (8-OHdG). Data are shown as mean ± standard error and differences were compared by paired or unpaired t test as appropriate.
There were no differences in clinical outcomes at follow-up. Flow mediated vascular reactivity increased by 37% (P= 0.20) and 109 %(P<0.01) in control and wine groups, respectively. Total Cholesterol /HDL ratio was significantly decreased by approximately 21 % in both groups (p< 0.04). Plasma total antioxidant capacity increased significantly only in the wine group, from 273±20 to 330±15 µM for TAR (P<0.03) and 1219±82 to 1449±63 µM for FRAP (P<0.001). Oxidative DNA damage in controls decreased from 13.1±1.1 to 10.0±1.0 (P<0.003) whereas with wine, it decreased from 13±0.8 to 5.6±0.7 per 10^5 guanosines (P<0.001; p=0.002 *vs* control).
Conventional therapy after an ACS managed with PTCA was useful to reduce lipid alterations and decrease DNA damage, whereas it did not increased significantly brachial reactivity. The addition of moderate red wine consumption to conventional therapy increased plasma antioxidant capacity and decreased DNA damage, which may be related with a significant improvement in endothelial function.

INHIBITION BY POLYPHENOL ANTIOXIDANTS OF OXIDIZED LDL INDUCED ENDOTHELIN-1 SECRETION IN ENDOTHELIAL CELLS

Miranda-Rottmann S[1], Martin-Nizard F[2], Briand O[2], Furman C[2], Fruchart JC[2], Duriez P[2] and Leighton F[1]. [1]Cellular and Molecular Biology Department, Faculty of Biological Sciences, Catholic University of Chile, Santiago, Chile and [2]Institut Pasteur, INSERM U545, Lille, France

Enhancement of endothelial function by moderate wine consumption has been attributed to protection of nitric oxide (NO), a free radical, by phenolic antioxidants. However the vasocontrictor endothelin 1 (ET-1) has now been shown to mediate the most important protective effect of wine on endothelial function. To evaluate the role of ET-1 we studied the effect of the phenolic compounds quercetin (Q) and ellagic acid (EA) on lipoprotein-induced secretion of ET-1 in endothelial cells. Both Q and EA inhibited lipoprotein-induced ET-1 secretion. NO synthase (NOS) activity was inhibited by oxidized lipoproteins and by phenolics. These results show that lipoprotein-induced endothelial dysfunction and wine beneficial effects might result from changes on ET-1 production. The contradictory effects of phenolics on NOS activity will be discussed.
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TRANSDUCTION MECHANISMS OF ENDOTHELIAL ADRENOCEPTORS AND NUCLEOTIDE RECEPTORS

Huidobro-Toro JP, Poblete MI, Buvinic S and Germain A. Faculty of Biological Sciences and Faculty of Medicine, Catholic University of Chile, Santiago, Chile

To assess the role and putative physiology of endothelial adrenergic and nucleotide receptors and their transduction mechanisms, we perfused either the rat arterial mesenteric bed or human placental cotyledons with selected ligands for these receptors. We monitored changes in perfusion pressure and the luminal release of nitric oxide (NO, measured by chemiluminescence) and tissue production of cGMP as the NO-evoked second messenger. Selective β-adrenoceptor ligands or P2Y receptor ligands caused a concentration-dependent vasodilatation coupled to a rise in endothelial NO and tissue cGMP production. Perfusion of the rat arterial mesenteric bed with 1 nM adrenaline, isoproterenol, terbutaline, CGP 12177 or BRL 37344 released 293±30; 155±15; 180±15; 207±23; 66±14 and 72±14 pmol NO respectively (n= 4-8). The rise in tissue cGMP following 1nM adrenaline or BRL was 21±2.2 and 21±5.5 pmol cGMP/g, respectively, these values were markedly increased following phosphodiesterase inhibition. The BRL-induced vasodilatation was antagonized with 10-100 nM SR 59230A, a selective β_3-adrenoceptor antagonist, but not 10-100 nM propranolol. Parallel experiments revealed that both 2-MeSADP or UTP vasodilatate this bed raising the luminal release of NO, and the tissue cGMP, consonant with selective $P2Y_1$ and $P2Y_2$ receptor activation. PCR studies confirmed the presence of mRNA for β_1, β_2 and the β_3-adrenoceptors and the Y_1 and the Y_2 nucleotide receptors in endothelial cells. Likewise, the perfusion of isolated human cotyledons with subtype selective nucleotide agonists evidenced the presence of endothelial Y_1 and Y_2 nucleotide receptors, which vasodilate via a mechanism coupled to the NO/cGMP pathway. Perfusion with 100 nM 2-MeSADP or 10 mM UTP evoked the net release of 146±50 and 125±15 pmol NO and a rise in 412±77 and 570±89 pmol cGMP/g, respectively. PCR confirmed the endothelial expression of Y_1 and Y_2 receptors. Altogether, the present results highlight the role of β-adrenoceptors and several nucleotide receptors in blood flow distribution. Their modulation in health and disease remains challenging.
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8. Mediterranean Diets

ROLE OF EDIBLE WILD PLANTS RELATIVE TO OMEGA-3 FATTY ACIDS AND ANTIOXIDANTS

Simopoulos AP. The Center for Genetics, Nutrition and Health, Washington, D.C., USA

Human beings evolved on a diet that was balanced in the omega-6 and omega-3 polyunsaturated fatty acids (PUFA) and high in antioxidants. Edible wild plants provided alpha-linolenic acid (ALA) and higher amounts of vitamin E and vitamin C than cultivated plants. In addition to the antioxidant vitamins, edible wild plants were rich in phenols and other compounds that increased their antioxidant capacity. Studies have shown that cultivated plants contain lower amounts of vitamins and essential minerals, and that their content has decreased by one-third over the past 50 years.

The diets of Western countries have contained increasingly larger amounts of linoleic acid (LA), which has been promoted for its cholesterol-lowering effect. It is now recognized that dietary LA favors oxidative modification of low density lipoprotein (LDL) cholesterol, increases platelet response to aggregation, and suppresses the immune system. In contrast, ALA intake is associated with inhibitory effects on the clotting activity of platelets, on their response to thrombin, and on the regulation of arachidonic acid (AA) metabolism. In clinical studies, ALA contributed to lowering of blood pressure. A prospective study showed that ALA is inversely related to the risk of coronary heart disease in men.

Dietary amounts of LA as well as the ratio of LA to ALA appear to be important for the metabolism of ALA to long-chain omega-3 PUFAs. ALA is not equivalent in its biological effects to the long-chain omega-3 fatty acids found in marine oils. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are more rapidly incorporated into plasma and membrane lipids and produce more rapid effects than does ALA. Relatively large reserves of LA in body fat, as are found in vegans or in the diet of omnivores in Western societies, would tend to slow down the formation of long-chain omega-3 fatty acids from ALA. Therefore, the role of ALA in human nutrition becomes important in terms of long-term dietary intake. One advantage of the consumption of ALA over omega-3 fatty acids from fish is that the problem of insufficient vitamin E intake does not exist with high intake of ALA from plant sources.

INTERACTION OF OLIVE OIL PHENOL ANTIOXIDANT COMPONENTS WITH LDL

Lamuela-Raventós RM. Departament of Nutrition and Food Science, CÈRTA, Pharmacy Phaculty, University of Barcelona, Spain

Intervention studies with wine extract, rich in phenolic compounds, have shown that phenolic compounds could decrease LDL oxidation. Phenolic compounds have been shown to inhibit LDL oxidation in vitro and ex vivo; however they are hydrosoluble compounds while LDL is a lipoprotein. Analysis of these compounds in LDL's by HPLC is necessary to demonstrate their binding capacity to lipoproteins. The aims of our study were:

To develop a method that allowed the analysis of these compounds in LDL's.

To evaluate the effect of a phenolic extract from virgin olive oil on LDL in vitro.

To study the effect of the ingestion of food rich in phenolic compounds on LDL.

We developed and validated a solid phase extraction method (SPE) that allowed us the purification of LDL sample and the analysis by HPLC. This methodology allowed us to demonstrate the binding capacity of tyrosol, one of the main phenolic compounds in olive oil, to LDL in vitro. In the intervention dietary study with volunteers food rich in phenolic compounds affects LDL composition. After one week of olive oil consumption there was an increase in oleic acid ($p<0.05$), vitamin E ($p<0.05$), phenolics ($p=0.021$) and lag time ($P<0.0001$) and a decrease in the maximum amount of dienes ($P=0.05$) and oxidation rate ($p=0.05$). Our results support the idea that ingestion of food rich in phenolic compounds could protect LDL from oxidation.

CATECHIN AND PROCYANIDINS IN MEDITERRANEAN DIETS

Teissedre PL. Université Montpellier 1, Faculté de Pharmacie, Département d'Oenologie, Montpellier Cedex 5, France

A great many epidemiological studies indicate that a diet rich in flavonoids from vegetable and fruit intake appears to be inversely related to coronary heart disease (CHD) mortality. Regular moderate consumption of wine can contribute to this phenomenon. Flavonoids in wine and food have been shown to be antioxidant and anti-aggregant in vitro and could indeed help protect against coronary disease. Thus, flavonoids may partly explain the protective effects of the Mediterranean diet, rich in vegetables, fruits and wine against CHD. The first step in evaluating this hypothesis is to create a catechin-food composition table. For red wine, the consumption of 180 mL for which the mean catechin and procyanidin concentration is 557.9 mg/L gives a mean daily intake of 100.4 mg of these compounds. This reasoning applied to each type of wine (red, white, rosé) for regular (daily), and moderate (180 mL) consumption gives estimations of catechin and procyanidin intake of 100.4 mg for red wines, 2.7 mg for white wines and 3.1 mg for rosé wines. A second step consists in determining to what extent each of these foodstuffs contributes to antioxidant flavonoids in the blood. Which type of diet contributes most to plasma concentration of (+) catechin. Our recent study demonstrates that in 182 subjects consuming Mediterranean foodstuffs, the highest concentration level of (+)-catechin in plasma was observed when wine, fruit and vegetable were consumed. Among these vegetal foodstuffs, red wine appears to be the most effective in producing this effect in a sample of free-living population in the south of France. If as reported, antioxidant flavonoids, especially catechin and procyanidins, have a significant protective effect against CHD, red wine and some fruits and vegetables, owing to their flavonoids, may provide the highest protection among all the Mediterranean foodstuffs which have been tested.

WHITE WINE AND RED WINE ON CARDIOVASCULAR RISK FACTORS IN A DIETARY INTERVENTION STUDY

Leighton F, San Martín A, *Castillo O, *Pollak F, Pérez DD, Strobel P, Urquiaga I, Urzúa U, Díez MS, Foncea R, *Cuevas A, *Germain A, *Rozowski J and *Mezzano D. Faculty of Biological Sciences and *Faculty of Medicine, Universidad Católica de Chile, Santiago, Chile

An intervention study in young healthy male volunteers was made to evaluate the influence on cardiovascular risk factors of high fat diets (40 %) with low (0.9) and high (5.3) MUFA/PUFA ratios, and the supplementation of these diets with white wine, red wine, and fruits and vegetables. Two groups of 22 volunteers received either one of the specially prepared diets. For an initial 4 weeks period they received only the diet. Then in 3 sequential periods of 3 weeks each, they received isocaloric supplementation with white wine (WW), Chardonnay (250 ml/day), followed by red wine (RW), Cabernet Sauvignon (250 ml/day), and fruits and vegetables (F&V) (8 servings/day) in the final period. The main changes observed were: an increase in plasma HDL with both WW and RW and a less marked increase with F&V; a decrease in blood pressure particularly with F&V and WW, less with red wine, in fact WW reduced blood pressure more than RW; a striking elevation of endothelial function in the period with RW; and a marked decrease in oxidative DNA damage in the period with RW, with lesser but statistically significant reductions after F&V. Some wine polyphenols were elevated in plasma during the RW supplementation period. In conclusion, moderate wine consumption and fruits and vegetables favorably modify cardiovascular risk factors, RW provides additional benefits probably due to its antioxidant properties and WW, in what constitutes an unexpected finding, showed a clear cut hypotensive effect. The WW hypotensive effect is now under evaluation applying a specifically designed experimental protocol.
(Part of these data was presented at XXVème Congrès Mondial de la Vigne et du Vin, Paris, 19-23 Juin, 2000)
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CHILEAN DIET: A MEDITERRANEAN-LIKE DIET?

Rozowski J. Department of Nutrition, Diabetes and Metabolism, School of Medicine, Catholic University of Chile, Santiago, Chile

Health indicators in Chile have shown relatively good results compared to other developing countries. Recently the World Health Organization published the Disability-Adjusted Life Expectancy (DALE) which analyses life expectancy adjusted for disability due to the prevalent diseases in each country. Countries like France, Spain, Italy, and Greece, which have a Mediterranean-style diet (MSD), showed an index of 73.1, 72.8, 72.7 and 72.5 years, respectively. Chile showed the highest DALE in Latin America (68.6 years), even higher than the Cuban one (68.4 years). In relation to cardiovascular mortality, Chile shows rates similar to those Mediterranean countries (164/100,000) and much lower than other countries like USA (223/100,000) or neighboring Argentina (277/100,000). Although many factors may be involved, Chilean diet has characteristics of a MSD. The traditional diet is composed of cereals (mainly bread), white meats (mainly chicken), potatoes, and a high amount of legumes (mainly beans and lentils). Traditional, preparations like beans with pasta (porotos con riendas), chicken soup (cazuela de ave), conger soup (caldillo de congrio), and others, do fold into the paradigm of the MSD. Nevertheless, although we have not had a national food intake survey in almost 20 years, smaller studies show that there seems to be an "occidentalization" of our diet,. This involves an increase in the proportion of fat calories, an increase in the consumption of read meats, and the adoption of a consumption typical of industrialized countries. For instance, Chile has one of the highest intake of soda per capita in the world, reaching approximately one can of soda/capita/day. Wine consumption, a feature of MSD, has decreased markedly from 40 l/capita/year in 1980 to 15 l/capita/year (consumption in Spain and Greece is about 35 l/capita/year, and in France and Italy 60 l/capita/year). Therefore, the changing in the pattern of food intake in the country shows an unfavorable trend. This presentation will discuss the principal characteristics of the Chilean diet, its comparison with MSD, and ways in which the changing trend in dietary intake can be reversed.

WINE DRINKING AND RISK OF CARDIOVASCULAR COMPLICATIONS AFTER RECENT AMI

De Leiris J and De Lorgeril M. Laboratoire Stress Cardiovasculaires et Pathologies Associées, Faculty of Medicine & Pharmacy, Batiment Jean Roget, University of Grenoble, France

Scientific data on the clinical impact of moderate alcohol consumption after a recent acute myocardial infarction (AMI) are limited and the specific effect of wine ethanol has not been studied. In survivors of a recent AMI, we analysed the association between ethanol intake and the risk of recurrence. The patients were classified according to the amount of ethanol that they consumed regularly during follow-up. Major prognostic factors, including the severity of the prior AMI and drug treatment, were recorded and included into the analyses. Only patients with at least two reliable assessments of drinking (and dietary) habits were included (n=437). The average ethanol intake was 7.6 % of the total energy intake, wherein wine ethanol represented 92 % of the total. Among these patients, 104 cardiovascular complications occurred during a mean follow-up period of 4 years. In comparison with abstainers, the adjusted risk of complications was reduced by 59 % (95 % confidence interval 17-80) in patients whose average ethanol intake was 7.7 % of the total energy intake (about 2 drinks/day), and by 52 % (95 % confidence interval 4-76) with an average ethanol intake of 16 % of energy (about 4 drinks/day). Thus, whereas moderate wine drinking was associated with a significant reduction in the risk of complications in this homogenous population of CHD patients, further studies are required to confirm the data, to define the clinical and biological profile of the patients who would most benefit from wine drinking after recent AMI, and to examine whether the relations found are due to ethanol or to other wine ingredients.

MEDITERRANEAN DIET, OCCIDENTAL DIET, AND WINE INTERVENTION STUDIES

Urquiaga I[1], Cuevas A[2], Guasch V[1], Pérez DD[1], Strobel P[1], San Martín A[1], Díez MS[1], Foncea R[1], Castillo O[2], Mizón C[2], Espinoza MA[1], Rozowski J[2], Maiz A[2] and Leighton F[1]. [1]Faculty of Biological Sciences and [2]Faculty of Medicine, Catholic University of Chile, Santiago, Chile

Diet is a key factor in the control and development of chronic diseases, including cardiovascular disease (CVD), cancer, diabetes, and others. Atherosclerosis and cancer are the main cause of death in industrialized countries. Since the Mediterranean basin populations show a lower mortality rate from all causes, as well as a low specific mortality from CVD, phenomena which could partially be explained by their dietary habits, the Mediterranean diet has often been proposed as a prototype or dietary paradigm

on which dietary recommendations for the prevention of CHD in populations may be based. In contrast, most other occidental developed countries show a high risk of chronic diseases, so their diet has been catalogued as inconvenient and a risk factor for chronic diseases. The diet of the people from the Mediterranean countries of southern Europe is characterized by a relatively high consumption of fish, white meat, olive oil, legumes, vegetables and fruits; by a lower consumption of red meat and animal fat and by a moderate consumption of red wine with meals. Conversely, Occidental diet, typically from the USA and some other continental and northern European populations, is characterized by high dietary intake of red meat, animal fat, dairy products and sugar, and decreased consumption of legumes, vegetables and fruits and, in several populations, of sea food. In terms of nutrients the Mediterranean diet is rich in monounsaturated fatty acids (MUFA), fiber and antioxidants, balanced in omega-6/ omega-3 polyunsaturated fatty acids (PUFA) and it is low in saturated fat (SFA). The Occidental diet is high in SFA and omega-6 PUFA, in refined or simple carbohydrates and poor in antioxidants and fiber. Oxidative stress, the consequence of a prooxidant imbalance among prooxidants and antioxidants in the human body, has been implicated during the last decade in the pathogenesis of chronic diseases and aging. Since antioxidants reduce or suppress oxidative stress, they could prevent the damage caused by reactive oxygen species (ROS) and decrease the risk of chronic diseases.

In this context, the benefits of Mediterranean diets have been attributed to a large consumption of antioxidants provided by fruit, vegetables and wine; and by the type of fat, especially MUFA and omega-3 PUFA from vegetables and fish. The damage potential of Occidental diets has been associated to a high intake of SFA and n-6 PUFA from red meat, dairy products and some vegetable oils. PUFA are susceptible to ROS and are oxidized to lipid peroxides, generating chain reactions which cause extensive damage to DNA, protein, carbohydrates and lipids.

In order to compare the effect of MD and OD, plus their supplementation with red wine, on biochemical, physiological, and clinical parameters related to atherosclerosis and other chronic diseases, we carried out an intervention study in humans. For 3 months two groups of 21 male volunteers each, received either MD or OD; during the second month of diet, red wine was added isocalorically, 240 ml/day. At days 0, 30, 60 and 90, clinical, physiological and biochemical parameters were evaluated. In this presentation, we analyze the results obtained in plasma antioxidants, plasma antioxidant capacity, plasma fatty acids and oxidative damage markers. Plasma vitamin C decreased significantly and vitamin E increased in the OD group, relative to the MD group. After wine supplementation a significant increase in plasma vitamin C and a decrease in vitamin E was observed in both groups. Total plasma antioxidant capacity (TAR, total antioxidant reactivity) was higher in the MD group than in the OD group. In both groups, wine induced a marked increase in TAR. Plasma fatty acid measurements in the OD group, compared to the MD group, did not show differences in SFA, but lower levels of MFA and w-3 fatty acids, and higher levels of PFA and w-6 fatty acids, with a higher w-6/w-3 ratio. Wine supplementation reduced MFA, and increased PFA in both dietary groups. SFA decreased and w-3 fatty acids increased during wine period, but only in the MD group. A possible residual effect of wine supplementation on all the fatty acid variables was shown. Oxidative DNA damage, measured as 8-OHdG levels in blood leukocyte DNA, was markedly increased by the OD yet, it was markedly reduced after wine supplementation, in both OD and MD groups.

CONCLUSIONS. These results support several conclusions: 1- OD induces oxidative stress; 2- MD enhances antioxidant defenses; 3- wine supplementation, both to OD and MD diets, markedly increase antioxidant defenses and decreases oxidative DNA damage. Fatty acid profiles and w-6/w-3 ratio showed better values in MD than in OD. Wine consumption improved the levels of SFA and w-3 fatty acids toward a lower cardiovascular risk.

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9. Cultural and Educational Aspects Related to Wine Consumption

SOCIAL AND SOCIETAL CONTROL IN WINE CONSUMPTION

Spawton A. University of South Australia, Adelaide, Australia

The use of societal controls to modify alcohol use and abuse is continually under review. A battery of measures have been developed to protect society and to reduce the social costs of alcohol abuse and misuse. This study is unique in its investigation of the importance and compliance of alcohol consumers to these societal control measures. The paper is a part of a wider national study of the Australian alcohol consumers undertaken in 2000. The study shows that consumers generally are both cognisant of societal control often due to risk of legal penalty if the control measure is broken, e.g. penalties for drink drive offences.

Consumers of alcohol do endeavour to comply with societal controls, however, even though these societal controls do modify behaviour to moderate consumption in a specific set of consumption circumstances, they do little to develop a culture of continuing moderate consumption as a matter of practice and lifestyle. In certain circumstances they may even contribute to alcohol abuse by encouraging excessive consumption behaviours either prior to or after a period of control - the "six o'clock swill effect" [1].

[1] **Author's note**: The 6 o'clock swill was a phenomenon unique to Australia up until the 1960's when the sale of alcohol was curtailed after 6 pm each working day. The effect was a rush from the workplace to the hotel (pub) where alcohol was consumed as rapidly as time would allow prior to 6 pm.

ALCOHOLIC BEVERAGE PREFERENCE AND RISK OF HEAVY DRINKING AND CIRRHOSIS

Grønbæk M. Centre for Alcohol Research, National Institute of Public Health, Copenhagen, Denmark

Recent studies have suggested that wine drinkers are at lower risk of death than beer or spirits drinkers. In a large longitudinal study from the Copenhagen area, we examined whether the risk of becoming a heavy or excessive drinker and the risk of developing alcoholic cirrhosis differed with different beverage choice. Compared with those who preferred wine, those who preferred beer tended to have increased risk of becoming heavy and excessive drinkers. Women who preferred beer had odds ratios of 1.14 (95 % CI = 0.87-1.50) for becoming heavy drinkers and 1.50 (95 % CI = 0.93-2.43) for becoming excessive drinkers. For men who preferred beer the ORs were 1.16 (95 % CI = 0.84-1.58) and 1.81 (95 % CI = 0.85-3.82). We further confirmed the increasing risk for cirrhosis with increasing alcohol intake, since individuals who drank more than 5 drinks per day had relative risks between 14 and 20 for developing cirrhosis compared with non or light drinkers. However, compared with individuals who drank no wine (relative risk set at 1.0), individuals drinking 16 % to 30 % wine of their total intake had a relative risk of 0.4 (95 % confidence limits, 0.3-0.6) and those drinking 51 % or more of wine had a relative risk of 0.3 (95 % confidence limits, 0.2-0.5) for developing cirrhosis. The finding that moderate wine drinkers appear to be at lower risk of becoming heavy and excessive drinkers may add to the explanation of the reported beverage-specific differences in morbidity and mortality. Further, our results suggest that a high intake of all three types of alcohol conveys an increased risk for cirrhosis, but that wine drinkers are at a lower risk than beer and spirits drinkers.

THE EFFECTIVENESS OF BRIEF INTERVENTIONS AMONG EXCESSIVE DRINKERS

Heather N. Centre for Alcohol & Drug Studies, University of Northumbria at Newcastle, Newcastle upon Tyne, UK

Brief interventions among excessive consumers of alcohol are not merely another form of treatment for alcohol problems but represent a new principle of intervention in the effort to reduce alcohol-related harm. They form part of a paradigm shift in the science of responding to drinking problems: the shift from a disease/ clinical perspective to a public health and welfare perspective. This paper will begin by describing this paradigm shift before considering the evidence relating to the effectiveness of brief interventions. Evidence for effectiveness, particularly in the primary health care and other medical settings, is strong. However, a range of research issues remain to be explored, including: (i) the need for more research conducted under naturalistic conditions ("effectiveness trials") in addition to that conducted under optimal research conditions ("efficacy trials"); (ii) more evidence on the longer-term effects of brief interventions: (iii) further examinations of the possible economic benefits associated with brief interventions; (iv) determination of the optimal forms and lengths of brief interventions for different sub-groups of excessive drinkers. These and other research issues will be discussed. Finally, despite strong evidence of effectiveness, difficulties have been encountered in persuading medical and other practitioners to implement brief interventions routinely in their work. The barriers to such implementation and how they might be overcome will be briefly explored.

MODERATION IN AUSTRALIA, POLICY AND ACHIEVEMENTS

Stockley C. The Australian Wine Research Institute, Glen Osmond, Australia

Alcohol has been consumed in Australia since European settlement in 1788. In 1998, approximately 60% of Australians consumed an alcoholic beverage at least once per week. The effects of alcohol on the human body are dose dependent, where the harmful effects of alcohol are generally observed only when alcohol consumption exceeds moderate consumption levels of 30 to 40 g of alcohol per day. The discovery that a J-shaped curve described the relationship between level of alcohol consumption and risk of cardiovascular disease was, however, only made in 1990 –cardiovascular disease is the leading cause of death in the western world. Thus prior to 1990, Australian public health policy focussed primarily on the harmful effects of alcohol consumption and the health benefits of a moderate level of alcohol consumption have only recently been recognised in public policy. This paper chronicles changes in Australian Federal government policy on alcohol since the initial draft *National health policy on alcohol in Australia* was presented to the Ministerial Council on Drug Strategy in 1987 to the

National Drug Strategy– a plan for action 2001 to 2003-2004 which was launched in July last year.

AN INTERNATIONAL POLICY PERSPECTIVE FOR RECOMMENDATIONS ON MODERATION

Holmgren E. Science and Policy Consultant, Alamo, California

Over the last decade scientific findings have confirmed that sensible drinking can be part of a balanced lifestyle for most adults who choose to enjoy alcohol beverages. Consistently, the public has been made aware of these study results.It is reassuring that social science reports in countries like the United States have confirmed that the overwhelming majority of those who choose to enjoy alcohol beverages do so moderately and responsibly. Coinciding with this heightened public awareness on moderation and its potential lifestyle benefits, a reemerging alcohol policy debate is trying to identify the most appropriate messages to the public at large. Public health leaders call for detailed educational messages because of the remaining concerns about possible increased alcohol abuse problems if scientific findings were incorrectly or incompletely presented to the respective populations. Governmental and private policy organizations have provided somewhat varying guidelines with respect to the best advice and most emphasize the importance of individual advice by healthcare providers. A review of major alcohol inclusive guidelines such as the United States Dietary Guidelines for Americans, the United Kingdom's Sensible Drinking Guidelines and the Australian Alcohol Guidelines reveals cautious but balanced advice that address both potential risks and positive health effects. Based on these international perspectives, a summary *Education Message on Responsible Enjoyment* conveys the key principals that are intended to foster responsible behavior. This Message is based on conclusions of major medical research findings and details on who should or should not drink from official statements around the globe. This Message condemns underage and binge drinking, highlights different consumption levels for men, women and the elderly, and explains that consumption is not recommended when pregnant, while taking certain medications or operating any machinery. Based on the current international policy perspective, this summary guideline stresses moderation around a well balanced and nutritionally sound lifestyle for any adult who chooses to enjoy wine, beer or spirits. This is also supported by the various healthy traditional dietary concepts such as the Mediterranean Pyramid developed by Oldways Preservation and Exchange Trust in close cooperation with Harvard School of Public Health. These very sensitive and educational messages have been supported in principal by the industry, private and governmental sectors alike and continued outreach will serve the public health policy goals around the world in that education should further contribute to responsible consumption behavior by most individuals who drink for the enjoyment and enhancement of everyday living.

NEW APPROACHES FOR THE PHARMACOLOGICAL TREATMENT OF ALCOHOLISM.

Israel Y and Karahanian E. Department of Pharmacological and Toxicological Chemistry, Faculty of Chemical and Pharmaceutical Sciences and Millennium Institute CBB University of Chile and Thomas Jefferson University

Disulfiram, a time-honored drug for the treatment of alcoholism, has a number of side effects that reduce compliance with medication-taking. Nevertheless, when disulfiram administration is supervised, the anti-alcohol effects are excellent and exceed those of supervised naltrexone, recently approved by the FDA. Disulfiram has also been shown to be a valuable adjunct to treatment with acamprosate, a new drug that should stand the test of time. East Asians in Japan, Korea and China present a natural endogenous version of disulfiram, a mutation in the aldehyde dehydrogenase gene, which renders the enzyme inactive and markedly protects against alcoholism those who carry the mutation. We have investigated the use of antisense oligonucleotides, a new type of gene therapy drugs that bind to mRNA and destroy the message. Stable antisense oligonucleotides against aldehyde dehydrogenase mRNA were found to markedly reduce the levels of aldehyde dehydrogenase, to elevate acetaldehyde levels when administered in conjunction with ethanol and to produce a marked aversion to ethanol in rats. The aversion is the same order of that generated by very large doses of disulfiram. Present studies are aimed at delivering genes that manufacture the antisense oligonucleotides in the body, which are expected to last for one to two years after a single dose. Studies with isolated liver cells are also promising and will be presented.
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ADDICTION AND MODERATE CONSUMPTION ARE THEY RELATED?

Seijas D. Department of Psychiatry, Faculty of Medicine, Catholic University of Chile, Santiago, Chile

The subject of moderate alcohol consumption is a key issue in public health. To achieve control of consumption it is necessary to address the problem of addiction, a difficult but very relevant task.
There is a long history of wine in our culture, from the Biblical stories to Prohibition in the USA, and to the excessive consumption subculture observed in Chile, a problem focalized nowadays in alcohol consumption by youngsters. At least in part this process would be the consequence of a loss of cultural values, a phenomenon affecting society in a globalized and demanding civilization.
Everybody knows that alcoholism is a sanitary and economic problem, yet one hundred years of consumption in Chile have not led to a change in the situation.
How to improve the social interaction with regard to alcohol consumption habits? The answer seems to be not prohibition but the generation of healthy lifestyles, that promote responsible alcohol consumption among those with low risk for addiction.
There are several drinking patterns, from abstinence, to occasional, normal or excessive consumption, all of these related to the concept of controlled drinking.
The difference between addiction and dependency is important in the present analysis, and the concepts of compulsivity or disease lead to different strategies of action. For this problem, we recommend the study of DSM IV or ICD 10, from the WHO.
Since dependency is a late phenomenon, affecting a small though important fraction of the persons that drink (5 % in Chile), it is worthwhile to explore preventive

approximations to this problem. This is what has been attempted in Chile through the definition of normal use of alcohol. This would favor a controlled alcohol consumption which would not be damaging, and conversely, could be associated to health benefits provided certain limits are respected.

The definition of controlled consumption is not easily established, but it is necessary to define objective and subjective criteria for its analysis.

With regard to the units of controlled consumption in Chile, until the 80s, it was one liter of wine per day (or the equivalent for other beverages) a largely excessive definition. In the UK, the recommendation is 14 alcohol units/week for women and 21 for men (13 grams of ethanol per unit in the UK). In the USA the recommendation is 3 x 4 (3 drinks per session, four drinking sessions per week).

Implementation of limits and consumption styles should be reinforced by teaching of controlled consumption at home, considering that today youngsters are exposed to extensive consumption pressure in groups of young people.

A typical example in this general context is the relationship of Mapuches and their culture with alcohol. Among them, the socio-cultural degradation to which they have been exposed has led to a higher risk of addiction and medical and psychosocial problems.

It is essential to be able to explore the risk and protection factors which specific conditions and consumption patterns might impose on the population, in order to prevent rather than endeavour to revert the consequences of excessive alcohol consumption.

In order to answer the question whether moderate consumption leads to dependency, it should be considered that there is no evidence indicating that dependency is a necessary outcome (with the exception of the known higher risk in the family of alcoholics).

It is interesting to consider that a recent investigation shows that moderate wine consumers have a smaller risk of excessive consumption and, eventually of dependency. Moreover, in beer consumers it has been shown that the risk of becoming heavy or excessive drinkers is greater.

Once dependency is established, is it possible to allow moderate consumption? When dependency is moderate to severe, this is not possible according to several authors (Davis, Edwards, Rand Report, etc.) but there is a proportion of the low dependent drinkers where training in moderate consumption appears valuable, since it is easier to obtain adherence to moderate consumption than to total abstinence.

This is a subject of great importance in Chile, whose exploration and study could lead to a real modification of the negative statistics and consequences of alcohol consumption.