ABSTRACTS OF THE POSTER PRESENTATIONS

INHIBITION OF ANGIOTENSIN CONVERTING ENZYME BY COCOA PROCYANIDINS.

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Angiotensin-converting enzyme (ACE) is a key enzyme in the regulation of the homeostasis of blood pressure. It converts the inactive protein angiotensin I into the biologically active angiotensin II which has vasoconstrictive activity. Many polyphenols and polyphenol-containing plants have been reported to inhibit ACE activity. Catechins and its oligomers (procyanidins), isolated from cocoa, were examined for their ability to inhibit the ACE. This inhibition was characterized (Km. Ki, Vmax) evaluating the enzyme activity by two kinetic assays, that used as substrates furylacryloylphenylalanylglycine (FAPPG) and hippuril-histidyl-leucine (HHL). Hydrolysis of FAPPG results in a decrease in absorbance at 340 nm and hippuric acid was quantified by HPLC with UV detection at 280 nm. Catechin and procyanidin fractions were able to inhibit ACE activity, in a dose-dependent manner. At a concentration of 2.5 μM, epicatechin, catechin, and dimer inhibited 15, 10 and 18 %, respectively. A similar effect was observed when plasma was supplemented with 1 mM catechins or procyanidins. No significant influence of the procyanidin oligomerization degree was observed. The inhibition was not related to a zinc chelation. The results demonstrated that flavan-3-ols and related procyanidins could have an inhibitory effect on ACE activity. The physiological relevance of that inhibition will be discussed in terms of bioavailibility and enzyme structure
Supported by UBA (B054 and B042). Procyanidin samples were kindly provided by Masterfoods Inc., Hackettstown, NJ,USA.

SYSTEMIC OXIDATIVE STRESS MAY NOT BE ASSOCIATED WITH ALCOHOL CONSUMPTION IN HUMANS

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The postulated association between alcohol consumption and oxidative stress is not well known. Therefore, we studied urinary 8-hydroxydeoxy-guanosine (8-OHdG, a biomarker of oxidative DNA damage) excretion in healthy male volunteers according to their alcohol intake. The study population consisted of 205 males aged 18-60 years (58 teetotalers, 76 moderate drinkers, and 71 heavy drinkers or alcoholics) with well documented drinking and smoking habits. There were no significant differences in the urinary 8-OHdG, adjusted for age and BMI, between teetotalers (14.3± 5.5 ng/mg creatinine), moderate drinkers (15.6± 8.0), and heavy drinkers (15.4± 9.5, p=NS). There was no association between urinary 8-OHdG and erythrocyte mean cell volume, γ-glutamyl transferase, or smoking. There were no significant differences in autoantibody titres against oxidized LDL and total antioxidant capacity of LDL ($TRAP_{LDL}$) between the groups. These data suggest that the amount of systemic oxidative stress may not be associated with alcohol consumption.

ANALYSIS OF NATURAL PHENOLIC ANTIOXIDANTS PRESENT IN FOOD

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Vegetables constitute an integral part of the human diet and posses numerous beneficial effects against chronic diseases. These properties have been associated to the presence of phenolic compounds. In the present study the phenolic content in food was determined. Food came from an intervention study performed by our group. The study consisted of two fatty acid rich diets divided into four isocaloric groups: diet, diet plus white wine, diet plus red wine and diet plus extra fruits and vegetables. Food was frozen, homogenized and extracted with 70 % acetone and total phenols were measured by Folin and were expressed as gallic acid equivalents. Diet supplied 297.8 ± 22.4 mg/day of total phenols; when supplemented with white wine, a small increase was observed (353.3 ± 22.4 mg/day). During supplementation both with red wine and with fruits and vegetables, a significative increase with respect to basal values was observed (853.8 ± 22.4 $p<0.001$ and 512.8 ± 46.4 $p<0.01$ respectively). Total phenols were significatively higher during supplementation with red wine in comparison to that of fruits and vegetables $p=0.014$. Additionally, several compounds were identified by HPLC chromatography with DAD and electrochemical detectors: chlorogenic acid, ferulic acid, myricetin, quercetin and apigenin. Our results showed a positive correlation of the identified phenols with the values of total phenols, in plasma and urine, for the volunteers of the intervention study.
(PUC-PBMEC 1999-2002)

HYDROXYCINNAMIC ACIDS ARE NOT THE PHENOLIC COMPOUNDS OF RED WINE THAT PREVENT EARLY AORTIC ATHEROSCLEROSIS IN HYPERCHOLESTEROLEMIC GOLDEN SYRIAN HAMSTERS

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The effects of a red wine phenolic extract (PE) on plasma lipoproteins and early atherosclerosis were studied on hamsters. Thirty two hamsters were divided into 4 groups of 8 and fed an atherogenic diet for 8 weeks. They received by force feeding 7.14 mL/(d.kg body) of PE in 12 % ethanol (E+PE) or PE in water (W+PE), mimicking a moderate consumption of red wine or alcohol-free red wine (PE was given at 30.4 mg/(d.kg body), or 12 % ethanol (E-PE) or water (W-PE) as their respective controls. Plasma cholesterol and triglyceride concentrations were lower in groups that consumed PE. The decrease in plasma Apo B concentration was mainly due to PE and was significantly lower in Group E+PE vs. Group E-PE (-7.5 %) than in Group W+PE vs. Group W-PE (-40 %). No difference was

seen for Apo-A1. PE significantly increased plasma antioxidant capacity by 9 % in Group E+PE and 18 % in Group W+PE versus their respective controls. Liver glutathione peroxidase activity was increased by 67 % in group receiving PE in water in comparison with that given water; there was no effect when PE was given in ethanol relative to its control. Aortic fatty streak area (AFSA) was significantly reduced in the groups receiving PE in ethanol (-32 %) or PE in water (-29 %) in comparison with their respective controls. Ethanol significantly reduced AFSA by 60 % (Group E-PE vs. Group W-PE) or 62 % (Group E+PE vs. Group W+PE). In a second experiment, three groups of 8 hamsters were fed the atherogenic diet for 8 weeks. They received by force feeding 7.14 mL/(d.kg body) of sinapic acid in water (W+SA; 14.3 µg) or caffeic acid in water (W+CA; 186 µg) mimicking a moderate consumption of alcohol-free red wine; a third group received chlorogenic acid in water (W+CL; 1.46 mg) mimicking the consumption of one apple/day for a 70-kg human to the equivalent for the daily weight of hamsters. No significant difference was observed between the three groups for plasma cholesterol, HDL-cholestserol, triglyceride, Apo-A1 and Apo-B concentration. The hydroxycinnamic acids only significantly increased plasma antioxidant capacity by 29 % but were not able to reduce AFSA. These data suggest that ethanol is a complementary component of phenolics in the benefits of red wine for hamsters and that chronic ingestion of PE in ethanol prevents the development of atherosclerosis through several mechanisms. At the time of moderate consumption of red wine, ethanol can improve the effects of phenolic compounds. However, alcohol-free red wine appears to be a very good alternative to red wine. Moreover, phenolic acids did not seem to be the components involved in the observed reduction of AFSA by PE.

CONCOMITANT CONSUMPTION OF WINE AND EDIBLE OIL DOES NOT INFLUENCE THE PEROXIDATION STATUS OF CHYLOMICRONS.

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The aim of this study was to describe the lipid peroxidation status of chylomicrons (CM) at peak concentration following an oral load of edible oil rich in PUFA, with and without wine. A conventional oral fat tolerance test was adapted to include PUFA in oil as a milkshake. Healthy volunteers ingested the milkshake randomly, with or without wine. Blood was taken and CM were separated. The lipid concentrations were determined for standardisation as reference mass for lipid peroxidation. The peroxidation status of the CM and oil was described by conjugated dienes (CD), lipid hydroperoxides (LOOH) and thiobarbituric acid reactive substances (TBARS) in the absence and presence of butylated hydroxytoluene (- or + BHT). The CM were also subjected to oxidative stress to determine the lag phase by CD, although it was established that LOOH gave similar results. The PUFA oil contained 15.4mmol/g of CD, 2.4mmol/g LOOH, and TBARS 13.2mmol/g (-BHT) and 0µmol/g (+BHT). The peroxidation status of the CM with and without wine (2.5 ±1.0 and 2.3 ±0.8µmol/g respectively), for CD, was not significantly different, P=0.5. The CD and TBARS (-BHT) content of the PUFA oil was higher than that of CM (P=0.02). The lag times of CM with and without wine were not significantly different: 70 ±18 versus 80 ±23min, P=0.4, nor were the AUC by 26 hours: 1.92 ±0.5 e+4 versus 1.88 ±0.5 e+4 µmol.min/g lipid, P=0.8. We conclude that compared with ingested oil, the CM peroxidation status except for LOOH is lower. There appears to be no protection to ingested oil processed into CM by concomitant consumption of wine. However, the potential health benefits of the antioxidant capacity of wine could be mediated through other mechanisms.

MARINATING BEEF WITH RED WINE MAY PROTECT AGAINST LIPID PEROXIDATION DURING COOKING.

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The aim was to determine whether red wine has a protective influence on the peroxidation of lipids in beef by marinating overnight, as dietary peroxidised lipids may influence atherosclerosis. Standardised portions of beef purchased in a supermarket were marinated in red wine at 4C. Twenty-one samples of marinated and non-marinated meat were analysed raw or cooked in a microwave for reproducible conditions. One gram was homogenised before extraction. Extracts were tested for conjugated dienes (CD), lipid hydroperoxides (LOOH) and thiobarbituric acid reactive substances (TBARS) (- and + BHT), as well as lipid content (triglycerides and phospholipids) for standardisation. Per mg lipid, raw meat contained the following: CD 116 ±44, LOOH 48 ±68, TBARS (-BHT) 25 ±22 and TBARS (+BHT) 7 ±6 nmol. Marinating altered the CD (84 ±25)(P=0.02) but did not significantly alter the LOOH or TBARS (+ and – BHT), (paired t test P>0.10). Cooking altered the peroxidation status of non-marinated meat as percentages: CD 3 ±42, LOOH 3 ±131, TBARS (-BHT) 66 ±126 and TBARS (+BHT) 186 ±545 whereas marinated meat displayed the following percent changes: CD –14 ±25, LOOH 32 ±244, TBARS (-BHT) 64 ±130 and TBARS (+ BHT) 174 ±465. The trend to lower CD by marinating was not significant (paired t test P = 0.070). Sub-analysis showed that the baseline CD status did not influence susceptibility. These results demonstrate a great variability in lipid peroxidation in meat and suggest that red wine may offer protection.

EFFECT OF PROCESSING ON ANTIOXIDANT PROPERTIES OF WHITE WINES

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This work reports the analysis of the antioxidant activity of 10 white wine samples obtained from three different processing procedures directed to enrich the wines with their endogenous polyphenols. A, B and C represent three different vinification processes: without (A) and with grape skin and seed contact (B and C). The difference between B and C was the time of contact with the skin and seed. White wine samples obtained from B and C processes contained increasing concentrations of total polyphenols, and, in parallel, exhibited increased capacity to scavenge free radicals in different model systems. The extent of oxidation inhibition of linoleic acid suspension and lecithin egg yolk vesicles exposed to a free radical source, was related to the wine polyphenolic content. It is concluded that processing white wine with grape skin and seed

contact leads to extraction of grape skin and seed polyphenols, producing a polyphenol-rich white wine with antioxidant characteristics similar to those of red wine.

INTAKE OF A MEDITERRANEAN-TYPE DIET BY ELDERS FROM PROVIDENCIA COUNTY, SANTIAGO, CHILE.

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The information available on food intake in the elders in Chile is restricted to individuals of low socioeconomic groups, but there is no data available on food intake in elders of higher income groups. We conducted a study to assess food intake in a group of elders from Providencia County, a middle-income community. A 3-day food registry for which volunteers were previously trained on how to fill it up was used to determine basal food intake. The intervention consisted in placing them on a Mediterranean-style diet (MSD) specially prepared and delivered to them during 8 weeks. When comparing several metabolic analytes in the individuals before and after the intervention we noticed no significant differences. When their basal diet was analyzed, we realized that they were already consuming a MSD. We present here an analysis of their basal diet vs. the intervention diet, compared to the diet typical of Mediterranean countries and the diet of poor elders in Chile. The intake of macronutrients and micronutrients was substantially higher than those reported previously in poor elders in Chile. Based on this study we conclude that the diet intake of elders of middle-income is similar to the MSD and it seems to be strongly dependent on income level.
Supported by PUC-PBMEC 2000.

ANTIDIABETIC ACTIVITY OF RED WINE POLYPHENOLS, ETHANOL OR BOTH, IN STREPTOZOTOCIN-TREATED RATS.

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A polyphenol extract from a Corbières (France) red wine (P, 200mg/kg), ethanol (E, 1ml/kg) or a combination of both (PE) was administered by daily gavage for 6 weeks to healthy control or streptozotocin-induced diabetic rats (180-200g). Treatment groups included C or D (untreated control or diabetic) and CP, CE, CPE (treated control) or DP, DE or DPE (treated diabetic). P treatment induced a reduction in body growth and food intake in both CP and DP groups. In DP, hyperglycemia was reduced when measured one hour after daily treatment, but not at sacrifice (no treatment on that day). The hyperglycemic response to oral glucose tolerance test (OGTT) and plasma insulin at sacrifice were impaired similarly in DP and D groups. In contrast, in DE or DPE, body growth was partially restored while hyperglycemia was reduced both during treatment *and* at sacrifice. In addition, the hyperglycemic response to OGTT was reduced and plasma insulin was higher in DE or DPE than in D animals, indicating a long-term correction of diabetes. When calculated individually, the lowering of hyperglycemia between start and end of treatment was significantly greater in DPE or DE than in D, and greater in DPE than in DE animals. In summary, our study shows that (a) a polyphenol extract from red wine (used at a "pharmacological" dose) reduces hyperglycemia while decreasing food intake and body growth, (b) ethanol ("nutritional" dose) administered alone or in combination with polyphenols is able to correct diabetes, and (c) the combination possesses additive effects on glycemic control. Our data also illustrate the differential effect of polyphenols when administered in the presence or absence of ethanol.

ETHANOL AND POLYPHENOLS (CAT, QUER) INCREASE EXPRESSION OF FIBRINOLYTIC PROTEIN mRNAS IN VIVO IN RAT AORTIC ENDOTHELIUM.

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Moderate red wine consumption reduces the risk for CHD. This cardioprotection may be due, in part, to increased endothelial cell (EC) fibrinolysis, induced by the combined and/or synergistic effects of ethanol and wine components (i.e. polyphenols). Fibrinolysis increased through the regulated expression of fibrinolytic proteins (t-PA, u-PA, PAI-1) will be expected to reduce the risk for thrombosis and CHD-related mortality. Effects of ethanol/polyphenols on t-PA, u-PA and PAI-1 mRNA expression were determined, *in vivo*, in a rat model. Male Sprague-Dawley rats, 250-300 g were gavaged with ethanol (final blood level, 0.05%) or individual polyphenols (catechin, 0.495 mg/kg; quercetin, 0.033 mg/kg body wt) or saline control. Rats were sacrificed after 6 hr, thoracic aortas removed/fixed and paraffin embedded. Changes in protein mRNA expression in aortic endothelium were assessed by in situ hybridization and RT-PCR. In situ hybridization showed that ethanol and polyphenols rapidly increased t-PA and u-PA and decreased PAI-1 mRNA expression, respectively, compared to controls. RT-PCR analyses showed ~3- to 4-fold increase in t-PA and u-PA mRNAs and a 55% reduction in PAI-1 mRNA. These results showed that ethanol and each polyphenol is rapidly absorbed and achieves a sufficient blood concentration to induce the rapid up-regulation of t-PA and u-PA and down-regulation of PAI-1 mRNA expression, in vivo. Induced changes will presumably result in increased EC fibrinolysis and hence afford cardioprotection.

MAPKINASES MEDIATE EXPRESSION OF FIBRINOLYTIC PROTEINS BY ETHANOL AND POLYPHENOLS IN HUMAN ENDOTHELIAL CELLS (ECS)

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Moderate alcohol consumption reduces the risk for CHD. This cardioprotection may be attributed, in part, to increased EC fibrinolysis. MAPK cascades include the mitogen-activated protein kinases, p38, c-jun NH2-terminal kinase (jnk) and the extracellular signal-regulated kinase (erk1/2) that activate transcription factors and gene transcription. To determine whether MAPK cascades were activated by ethanol and polyphenol ECs were incubated in the absence/presence of ethanol (20 mM) or polyphenols (cat, quer, 10 μM, 0-30 min) and MAPK activation assessed by Western blot analysis, using antibodies against

active forms of p38, jnk, and erk1/2. To determine which cascade regulates fibrinolytic protein (t-PA, u-PA and PAI-1) mRNA expression, ECs were pre-incubated with inhibitors of p38 kinase (SB-203580) or erks (U-0126) for 45 min, followed by inducers (20 mM, 0-8 hr). Total RNA was extracted and fibrinolytic protein mRNAs were analyzed by RT-PCR. Ethanol and polyphenols activated the p38, jnk and erk1/2 kinases. T-PA and u-PA mRNA expression was inhibited by SB-203580, indicating involvement of p38 kinase. Ethanol and polyphenols repressed PAI-1 mRNA in the presence of SB-203580, but not U-0126, indicating involvement of the erk1/2 kinases. These results indicate that the ethanol-/polyphenol-induced up-regulation of t-PA and u-PA and concurrent down-regulation of PAI-1 mRNA expression in cultured human ECs, occurs through the activation of multiple, distinct MAPKs cascades.

CONSUMER ATTITUDE TOWARDS FOOD CONTAINING DIETARY FIBER

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It is interesting to know the current attitude of consumers after the publicitary impact that promotes fiber consumption; with this purpose, Fishbein and Ajzen's theory was applied, which states that individual intention (I) is a good estimate of behavior (B), which depends itself on attitude and subjective norms.
B ª I = Attitude x W_1 + Subjective norm x W_2
A survey was administered to 587 adults which consisted of 45 questions concerning: attitude (4), beliefs (13), evaluation of beliefs (13), self-concept (3), perceived level of surveillance (2), subjective norm (1), motivation for compliance (3), normative beliefs (2), behavior (2), behavioural intention (2), test complexity (1). Significative differences were found in the perception of the importance of fiber in health, its physiological effect and its price. In the Metropolitan Region, Chile, beliefs about fibre are appraised differently than in the other regions. Mens' beliefs are different from those of women's (p£0.05). The main components analysis (MCA) for all consumers pointed out that total variation is explained by the first three indexes (56.6 %), being the first one related to health beliefs, the second one to appearance and price and the third one to flavor. The simple correlation for all the elements of the model is 0.75. Highly significative improvements for the attitude, subjective norm and self-identity components are produced when their relative weight is estimated. Normative beliefs produce a significative improvement in the predictive capacity of the model ($R^2 = 0.41$).
Fishbein M., Ajzen I. (1975) "Belief, attitude, intention and behavior. An introduction to theory and research". Reading MA: Addison-Wesley.

OTOBAPHENOL FROM *Virola aff. pavonis*: ANTIOXIDANT PROPERTIES, AGGREGATION AND INFLUENCE ON MITOCHONDRIAL FUNCTIONS.

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The lignan otobaphenol, (8R,8´R,7R)-4´-hydroxy-5´-methoxy- 3,4-methylendioxy-2´.7,8.8´-neolignan, was extracted from *Virola aff. pavonis* leaves, and its antioxidant properties were studied using the model of Fe^{3+}-ascorbate-induced lipoperoxidation of mitochondrial membranes based on the measuring of the oxygen consumption and malondialdehyde formation. The antioxidant activity of otobaphenol was compared with that of butylhydroxytoluene. At concentration 2.5 μM, both substances almost completely inhibited the lipoperoxidation for at least 10 min of incubation. At concentration 25 μM, otobaphenol essentially delayed the permeability transition of rat liver mitochondria induced by *tert*-butylhydroperoxide or Ca^{2+}, somewhat inhibited the state 3 respiration with succinate as substrate of oxidation, did not affect the state 4 respiration and the coefficient ADP/O, diminished accumulation of Ca^{2+} by mitochondria and essentially delayed ruthenium red-insensitive, uncoupler-induced release of accumulated Ca^{2+}. At a concentration higher than 75 μM, otobaphenol caused mitochondrial aggregation and was able to self.aggregate in the absence of mitochondria. The formed aggregates of otobaphenol were able to delay the permeability transition of subsequently added mitochondria. We suggest that the aggregates of otobaphenol and of other natural resins with antioxidant properties could be used as a powerful "antioxidant buffer" for various applications.
Support: project 1118-05-261-97 of Colciencias.

CU(II) INDUCED LDL OXIDATION. EFFECT OF RED WINE AND ITS FRACTIONS.

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Several studies have reported the antioxidant capacity of red wine in in vitro studies. In particular, it has been reported its protective effect in the Cu(II) mediated oxidation of LDL. In the present work we report results obtained regarding the antioxidant capacity of red wine and its fractions: neutral (mostly flavonoids), acid (mostly phenolic acids) and aqueous (mostly anthocyanins). We have found that the effect elicited by the addition of wine (or its fractions) depends on the state of the LDL and the time of the wine addition. Regarding red wine, it is strongly antioxidant when fresh LDL are employed, but it becomes a mild pro-oxidant when added at the beginning of the exponential oxidation phase. Similarly, it is a pro-oxidant when aged LDL are tested. The behavior of the different fractions is more complex. The aqueous fraction behaves as the whole wine. On the other extreme, the acidic fraction presents a pro-oxidant character, even when added to fresh LDL prior to Cu(II) incorporation. The behaviour of the neutral fraction depends on the amount added. At low concentrations, it behaves as pro-oxidant. At higher concentrations, when added at the beginning of the experiment, it behaves as antioxidant. The possible reasons for this different behavior will be discussed.
Acknowledgments: This work has been supported by FONDECYT (Project 100022 and doctoral fellowship to EP) and the Molecular Basis of Chronic Diseases Program (PUC).

Cu -INDUCED LDL OXIDATION ASSESSED BY LOW LEVEL CHEMILUMINESCENCE.

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It is important to find procedures that, in real time, allow following the in vitro oxidation of LDL, particularly at the early stages of the process. Among the promising techniques stands the measurement of the spontaneous chemiluminescence produced in the oxidation process. In the present communication we show that this is a suitable procedure to evaluate the effect of red wine components upon the rate of LDL oxidation. Furthermore, we discuss the origin of the observed chemiluminescence. By evaluating the time course of the process and the effect of several additives (BHT, epselen, chelators) we concluded that the measured light intensity is related to the accumulation of hydroperoxides and not to the steady state concentration of peroxyl radicals (Russel´s like mechanism).
Acknowledgments: This work has been supported by FONDECYT (Project 100022 and doctoral fellowship to EP) and the Molecular Basis of Chronic Diseases Program (PUC).

(-)-EPICATECHIN, (+)-CATECHIN AND A DIMERIC PROCYANIDIN INHIBIT NF-κB ACTIVATION IN JURKAT T CELLS.

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Transcription factor NF-κB is partially modulated by oxidants and its activation leads to the transcription of genes involved in T cell activation. In the present study we investigated the capacity of the flavanols (-)-epicatechin (E), (+)-catechin (C) and a dimeric procyanidin (dimer) isolated from cocoa to modulate NF-κB activation in Jurkat T cells. Cells were incubated for 24 h in the presence of variable concentration (4.3 to 17.2 μM) of E, C or dimer. At this time point, the number of viable cells was similar among the groups. Cells were subsequently treated with 50 ng/ml phorbol 12-myristate 13-acetate (PMA) for 4 h and total and nuclear fractions were isolated. PMA-induced the phosphorylation and degradation of the inhibitory peptide IκBα, events that were not affected by E, C or dimer. PMA treatment caused a 4.6-fold increase in NF-κB nuclear binding activity. The exposure of cells to E, C or dimer for 24 h, significantly decreased PMA-induced NF-κB nuclear binding activity. The maximum inhibitory effects were at 8.6 mM E (51 %), 17.2 μM C (50 %) and 17.2 μM dimer (75 %). Accordingly, the nuclear concentration of p50, a component of the active dimer, was positively correlated to the NF-κB nuclear binding activity (r: 0.83, $p < 0.006$). Results indicate that E, C and dimer can regulate the immune response partially by modulating the oxidants-responsive transcription factor NF-κB. Since the phosphorylation and proteasome-mediated degradation of IκBα were not prevented by E, C and dimer, the effects of these compounds have to be exerted at later steps in the activation cascade.
Dimer samples were kindly provided by Mars Inc., Hackettstown, NJ, USA.

IDENTIFICATION OF WINE POLYPHENOLS AND THEIR METABOLITES IN PLASMA AND URINE AFTER ACUTE INGESTION IN HUMANS.

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Wine polyphenolic antioxidants in moderate drinkers reduce the incidence of coronary heart disease, cancer and other pathologies. Their biological effects are probably dependent on their metabolism. In this work, the bioavailability of wine polyphenols was studied. Measurements were made for total antioxidant capacity (TAR, TRAP) and total polyphenols in urine. Wine polyphenols and their metabolites were identified by HPLC chromatography with electrochemical and DAD detection in samples of plasma and urine, before and after wine ingestion. Eleven volunteers consumed a diet poor in polyphenols during four days; on the fifth day, seven volunteers drank 375 mililiters of red wine (Cabernet Sauvignon), 1934 mg/L of polyphenols, and four volunteers drank an equal dose of white wine (Chardonnay), 310 mg/L of polyphenols. Some wine polyphenols were identified in plasma: catechin, epicatechin, gallic acid, protocatechuic acid, and metabolites of caffeic acid. An increase in the excretion of some polyphenols was observed, like ferulic acid, isoferulic acid, and also some non-specific biomarkers of polyphenol ingestion like hippuric acid, p-hydroxyphenylacetic acid and others. The concentration and excretion in the volunteers was higher after red wine than after white wine consumption. After red wine consumption the excretion of polyphenols gradually increased for a period of 6-13 hours to levels of 0.19 ± 0,08 mg polyphenols/mg creatinine. Up to 20 % of the phenols ingested was recovered as phenols in the urine.
(PUC-PBMEC 2000-2002)

POSTPRANDIAL INCREASE OF PLASMA LIPOPEROXIDATION PRODUCTS AND REDUCTION OF ANTIOXIDANT ENZYME ACTIVITIES

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Postprandial proinflammatory lipoperoxidation products and their defensive enzymes were measured in a group of 10 healthy men, first-degree relatives of coronary artery disease patients. Venous blood was drawn after 12 h fasting and 2, 4, 6, and 8 h after a fat-load test meal (53 g fat/m^2). Plasma lipid profile, low density lipoprotein-lipoperoxides levels (LDL-ROOH), phospholipids (PL), non-esterified fatty acids (NEFA), 8-*epi*-prostaglandin $F_{2\alpha}$ (8-isoprostane), insulin, platelet activating factor-acetyl hydrolase (PAF-AH) and serum paraoxonase activity (PON1) were performed. LDL-ROOH raised in parallel with triglyceride levels (TG), reaching significant peaks at 4 h ($P<0.005$); in a

subpopulation of "late responders" these values were still elevated at 8 h. The same trend showed an increase in total PL, lysophosphatidylcholine and sphingomyelin with a concomitant decrease of phosphatidylcholine ($P<0.01$) and other diacylphosphoglycerides. The plasma marker of in vivo inflammatory processes and oxidative stress, 8-isoprostane, showed a significant averaged peak ($P<.02$) which coincided with the LDL-ROOH peak in 80 % of the subjects studied. NEFA increased at 8 h ($P<.05$). The activities of the antioxidant enzymes progressively decreased, reaching a maximal reduction at 8 h: PON1 decreased by 18 % and PAF-AH by 26 % ($P< 0.03$) showing an inverse correlation with LDL-ROOH ($r = -0.53$, $P=0.006$). In addition to the lipid metabolic impairments, the postprandial period after a fat-load meal showed an imbalance towards the oxidative state which could be categorized as a critical atherogenic risk factor.

TOCOPHEROL CONTENT IN GRAPE SEED OIL AND ITS ANTIOXIDANT EFFECTS IN TERMOXIDATIVE CONDITIONS

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Grape seed oil is a by-product of the elaboration of wine which possesses various natural liposoluble antioxidants among which are tocopherols and tocotrienols, that have recently become interesting for their beneficial effects on human health. In consequence, it is important to study the mechanisms that affect the stability of the oil and the protective role of lipids in conditions of extreme temperature and presence of oxygen. For this purpose, 10 g of oil were subjected to forced oxidation by heating in tubes at 180 ºC for 6 hours. During the heating interval the decline of the tocopherols was determined by HPLC and oil oxidation was measured by the determination of polar compound formation by fractioning in a silica column. Results indicated that the oil contains mainly gammatocotrienol (γ-T3=205ppm), alphatocotrienol; (α-T3=195ppm); gamma tocoferol (γ-T=59ppm) and alphatocoferol (α-T=45ppm). The tocols suffered degradation in the following order: γ-T>γ-T3>α-T3>α-T (98 %, 70 %, 25 %, 5 % loss). The formation of polar compounds increased from 2.8 % to 18.6 %. It can be concluded that the loss of antioxidants initiates the decrease of the oil's stability and the formation of polar compounds in the oil.
Proyecto Fondecyt Nº 1011070.

A MULTI-ASSAY STRATEGY TO DEFINE ANTIOXIDANT CAPACITY

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The term antioxidant capacity (AC) is usually an interpretation of a simple experiment of inhibition of the reaction of an oxidant, that intents to mirror an in vivo situation. In this way, the AC has been defined for tissues, body fluids, foods, molecules, etc. The "simple experiments" used to define AC, ranged from very basic spectrophotometric techniques to highly sophisticated and expensive methodologies. However, the answer given by those methodologies lacks biological relevance, due to limitations in the characteristics of the target molecules, the reaction media, the kind of oxidizing agent, the complexity of the biological system, etc. Furthermore, the correlation between two methods to determine AC is seldom satisfactory. To be able to achieve a more accurate evaluation of the potential AC of a food, substance, etc., we propose the use of a composite parameter that includes results from different methods. Testing this approach, we used 6 experimental assays ranging from simple chemical to biological systems, using oxidant sources of oxygen and nitrogen active species. We will present the results obtained and the mathematical analysis of the data. From this initial results, we open to discussion on the validity of the concept of a multiple assay strategy to define AC, the experimental approaches that were used and which others should be included and the logic and validity of the developed equations.
Supported by UBA (B054 and B042)

RED WINE COMPONENTS AND CARDIOPROTECTION: EFFECTS ON HUMAN ENDOTHELIAL CELL (EC) FIBRINOLYSIS.

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Moderate red wine consumption reduces the risk for CHD-related mortality. This cardiovascular disease protection may be due, in part, to increased EC fibrinolysis induced by red wine components (i.e. ethanol and principal polyphenols) that will be expected to reduce the overall risk for thrombosis, CHD and MI. We examined effects of red wine, de-alcoholized red wine, polyphenols, ethanol and other antioxidants on rapid increased fibrinolytic activity in cultured human ECs. ECs were pre-incubated (15 min, 4 ºC) in absence/presence of ethanol (0.01-0.2 %, v/v) or red wine, de-alcoholized red wine, grape juice (all at 1:62.5-1:1.000 dilution) or polyphenols (cat, epicat, quer, resver, 0.001-10 μM) or antioxidants (probucol, BHT, DHB, trolox, 0.001-10 μM). Surface-localized fibrinolytic activity was measured by the direct activation of EC-bound ^{125}I-Glu-Pmg by endogenous EC-bound t-PA (10 min, 37 ºC). Activity was rapidly (<15 min) increased by red wine (~6-fold at 1:62.5 dilution) > ethanol (~4.5-fold at 0.20 % = ethanol at 1:62.5 wine dilution) > de-alcoholized wine (~4-fold at 1:62.5), compared to controls. Cat and epicat increased activity ~2.25-fold (at all concentrations), whereas quer and resver increased activity only ~1.5-fold at 10 μM. All other antioxidants and grape juice had no affect on fibrinolytic activity. Results indicate that ethanol and polyphenols act independently to increase EC fibrinolysis and that overall cardioprotection may be due, in part, to these multiple red wine components acting in combination or perhaps synergistically to increase EC fibrinolysis.

STUDY OF PHENOLIC COMPOSITION OF MERLOT AND CHARDONNAY COMMERCIAL WINES FROM FIVE VALLEYS OF CHILE

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Phenolic compounds are important components of wine. They not only contribute to the sensorial characteristics of wine, such as color, flavor and astringency, but also act as antioxidants, with mechanisms involving both free-radical scavenging and metal chelation.
The phenolic composition and concentration of wine depend among other factors on the variety of grape used for vinification, type of soil and environmental factors (light, temperature, rainfall, etc.), wine-making procedures and chemical reactions that occur during the aging of wine.
The objective of this work was to study the relation between phenolic composition and the origins (procedence) of different samples of commercial Merlot and Chardonnay young wines, from five valleys of Chile (Casablanca, Maipo, Rapel, Curicó and Maule), elaborated in the vintages 1998 and 1999. Physical and chemical analyses were carried out on 50 samples of each variety (five per valley and per year) in order to measure different parameters. They were: total acidity, pH, color (hue and colorant intensity), content of total phenols, total anthocyans and grade of polimerization of condensed tannins. Also some phenolic compounds (non-flavonoids, flavonoids and stilbens) were analysed by using High Performance Liquid Chromatography (HPLC-DAD) [1]. Regarding the results, it was posible to conclude that there exists a substantial influence of the procedence and also of the year of production on the phenolic content of the wines studied. This effect was better seen by using a discriminant analysis on the compouds studied by HPLC-DAD than through the global parameters measured.
1. Peña-Neira A, Hernández T, Estrella I, Suárez J (1998). Survey of *trans* resveratrol and *trans* resveratrol glicoside in Spanish red wines. Polyphenols in food, COST 916. European Community, Luxemburg, pp 221-225.
Acknowledgments for financial support: DID Project I-99-2; FONDECYT Project 1010224.

TOTAL ANTIOXIDANT CAPACITY IN ACUTE SEPSIS PATIENTS WITH SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS)

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Oxidative stress, an imbalance between the antioxidant defenses and the formation of free radicals, characterizes certain diseases or pathological conditions. One of these conditions is the Systemic Inflammatory Response Syndrome (SIRS) which compromises the functionality of several organs as a result of an acute and generalized septic shock. Depending on the gravity of this condition measured by defined sets of parameters like the SOFA or APACHE II indexes, life expectation can be estimated. Several studies have detected a progressive decrease of the antioxidant levels and an increase in lipid peroxidation, both at the beginning of SIRS and at later stages. OBJECTIVE: To determine the evolution of total antioxidant capacity (TAC), antioxidant levels and some parameters of oxidative stress, during the evolution of SIRS at early and late stages of evolution. RESULTS: Total antioxidant capacity measurements, TRAP, TAR and FRAP, showed a significant decrease, 43 % (p=0,002), 44 % (p=0,012) and 34 % (p=0,007), respectively, after 7 days of hospitalization in an adult intensive care unit (ICU and ITU). A marker of lipid peroxidative damage, TBARS, showed an initially high value, apparently generated during the beginning of the sepsis, 2.5 times greater than control values, and a further increase during evolution of the SIRS, 35 % ($p<0.05$). Nitrotyrosine in proteins was also evaluated. The liposoluble antioxidants and vitamin C did not show large changes during the intensive care period, yet their initial levels were approximately 50 % of control values. These values returned to normal levels in control measurements made after several weeks. CONCLUSION: The antioxidative system is severely compromised in SIRS with markedly reduced capacity already at the beginning of the intensive care period. In addition, a progressive increase of lipid peroxidation end products, TBARS, was observed in the plasma. These observations corroborate the severe oxidative stress condition inherent to SIRS. Decreased levels of TAC and plasma antioxidants would be the consequence of increased requirements. The TAC, the antioxidants and TBARS levels returned to normal levels months after the hospitalization. On the whole, these results raise two main questions: Which is the contribution of oxidative stress to the severity of SIRS? And, how could antioxidant defences in SIRS patients be enhanced during the acute phase of the disease?
(PUC-PBMEC-2000)

HOMOCYSTEINE INDUCES GENES THAT REGULATE MITOCHONDRIAL BIOGENESIS IN ENDOTHELIAL CELL. ROLE OF OXIDATIVE STRESS.

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Hyperhomocysteinemia (HH) has been associated with premature thrombotic vascular diseases. It has been suggested that the mechanism of homocysteine (Hcy) injury involves an oxidative stress increase. Experimental evidences suggest that mitochondrial reactive oxygen species (ROS) increase in damaged mitochondria. Recently, mitochondrial damage in endothelial cells from rats with diet-induced HH has been shown. However, the mechanism by which Hcy affects mitochondria is unknown. Mitochondrial biogenesis is the result of the coordinated expression of nuclear and mitochondrial genes. Mitochondrial transcription factor A (Tfam) and nuclear respiratory factor 1 (NRF-1) are codified in the nucleus and regulate transcription in mitochondria.
In this work, we propose that homocysteine alters mitochondrial biogenesis by a ROS-mediated mechanism.
To evaluate this hypothesis, we studied the expression of NRF-1 and Tfam in human umbilical vein endothelial cells (HUVEC) incubated with Hcy (100µM) by RT-PCR. Additionally, we studied the protective effect of polyphenolic antioxidants through pretreatment of cells with cathechin (15µM).
We found that Hcy stimulation resulted in a generation of ROS, induction of NRF-1 (3-fold) and Tfam (2-fold), compared to controls. These effects were inhibited by pretreatment with catechin, a polyphenol antioxidant.
These findings support the proposed role of ROS in mitochondrial biogenesis induced by homocysteine.
(PUC-PBMEC2002)

IDENTIFICATION AND QUANTIFICATION OF SOME POLIPHENOLIC COMPOUNDS IN CHILEAN RED WINE CABERNET SAUVIGNON.

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Red wines are a rich source of polyphenolic compounds. Mainly phenolic acids, flavonoids, anthocyanins and procyanidins. These compounds are powerful antioxidants and have special biological properties like: antithrombotic, anti-inflammatory, antiatherogenic and antiallergic properties. Studies have shown that moderate consumption of red wine is associated with a reduced risk of coronary heart disease and cancer. The present study utilized 6 commonly consumed Chilean cabernet sauvignon wines from 1998 to 2000, which were given to the volunteers of intervention studies in our laboratory. The total phenolic content of wine and its fractions were determined according to the Folin-Ciocalteu method. The wines had an average of 2280 mg/l to 3268 mg/l (gallic acid equivalents). Improved high-performance liquid chromatography (HPLC) procedures were used to investigate the flavonoids and phenolic acid content of the acid and neutral fractions. The most abundant compound found at the neutral fraction was catechin with 35.8 ± 15.1 mg/l and at the acid fraction was gallic acid with 29.0 ± 4.5 mg/l. Acid and base hydrolysis were also performed in the whole wine in order to quantify conjugated miricetin, quercetin, caffeic and p-coumaric acids. Anthocyanin content was analyzed by direct injection in HPLC and identified on the basis of its elution order, retention time and absorbance analysis. The HPLC anthocyanin content varied from 5.04 mg/l (cianidin-3-glucosido equivalents) in the oldest wine to 208.37 mg/l in the newest one.
(supported by PUC-PBMEC-2002)

GALLIC ACID INHIBITS ADVANCED GLYCATION END PRODUCTS MEDIATED ACTIVATION OF VASCULAR SMOOTH MUSCLE CELLS

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Advanced glycation end products (AGE) result from the nonenzymatic, covalent and irreversible modifications of proteins by carbohydrates. Plasma and tissue levels of AGE increase in individuals with diabetes and vascular diseases (VD) and are the main cause of death in these patients. VD involve the change of smooth muscle cells (VSMC) from a contractile to a secretory/proliferative phenotype. AGE are specific ligands for the RAGE receptor. Ligand-mediated activation of RAGE leads to the generation of reactive oxygen species (ROS). We assessed the effects of AGE on VSMC phenotypic change and the role of ROS in this process. Incubation with AGE, prepared with bovine serum albumin, (200mg/mL) significantly stimulated proliferation of primary culture rat VSMC (8.1±1.4 vs 4.8±1.3 cpm/mg prot) and rates of collagen biosynthesis (3.6±0.2 vs 2.5±0.2 cpm/g prot). These effects were abolished by preincubation of VSMC with gallic acid (GA, 20µM) or NADPH oxidase inhibitor diphenylene iodonium (DPI, 10µM). Significant increase of ROS production in AGE-treated VSMC was measured with dichlorofluorescein (4.0±0.4 vs 3.3±0.2 units). AGE-induced oxidative stress was accompanied by activation of NFkB, increased expression of heme-oxygenase-1. GA or DPI inhibited all these effects. Incubation of VSMC with AGE for 12 hours increased 3.7-fold mRNA for RAGE. GA abolished this increase. In DPI-treated VSMC, there were almost undetectable mRNA levels. In conclusion, ROS, possibly derived from NADPH oxidase, mediate AGE-induced proatherogenic phenotypic changes and RAGE expression in VSMC. Polyphenolic antioxidants apparently prevent this ROS-mediated VSMC activation.

THE TOTAL INTAKE OF ANTIOXIDANTS CORRELATES WITH PLASMA CAROTENOIDS.

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It is generally accepted that a diet rich in fruits and vegetables reduces the incidence of major diseases such as cancer, cardiovascular disease, diabetes, cataract and inflammatory diseases. Recommendations to increase fruit and vegetable consumption have therefore been implemented in most countries. Since the active compounds and the mechanisms involved in this protective effect have not been defined, the recommendations are that eating a variety of fruits and vegetables will provide the best protection. This is a safe principle but these general recommendations avoid the issue of which fruits and vegetables yield the best protection. A large and remaining challenge is therefore to identify the most beneficial dietary plants. To assess the biological role of dietary antioxidants we have quantitated the total amount of antioxidants in about one thousand different foods by the Ferric Reducing Antioxidant Power-assay (FRAP) method. These data have been used to calculate total antioxidant intake in a group of 61 adults aged 30 to 82 years (28 controls and 29 users of lipid lowering agents due to hyperlipidemia). Dietary intake was assessed by a seven-day weighed food recording. The contribution of various food groups such as coffee, tea, wine, cereals, fruits, vegetables, dairy products and meats will be presented. The correlations of the total antioxidant intake as well as antioxidant intake from various food groups and plasma antioxidants were also assessed. These data suggest that total antioxidant intake from various food groups is correlated to several plasma antioxidants.

THE INFLUENCE OF MODERATE RED WINE CONSUMPTION ON ANTIOXIDANT STATUS AND ON INDICES OF OXIDATIVE STRESS RELEVANT TO CORONARY HEART DISEASE IN HEALTHY VOLUNTEERS.

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Moderate red wine consumption is inversely related with CHD, an association popularised as the
'French Paradox' (Renaud & de Logeril, 1993). The protective effects have been attributed to phenolics contained in red wine originating from grape seeds, skins

and vine stems. Phenolics have been shown to act as potent antioxidants in in vitro studies by reducing damaging free radical reactions (Burns et al. 2000). A randomised, controlled study was performed with twenty-three healthy volunteers. Subjects in the intervention group consumed 375 mL/d red wine (Safeway's Bulgarian Cabernet Sauvignon, 1999, 12 % alc/vol, 2400mg/L polyphenols) for two weeks. Fasting blood samples were obtained at baseline and following the intervention period. Plasma antioxidant capacity and concentrations, conjugated dienes and TBARS produced in copper-oxidised LDL and fasting plasma lipids were determined.

	Intervention group (*n* 12)				Control group (*n* 8)			
	Baseline		Week 2		Baseline		Week 2	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
[a]AOX capacity (% Fremy's radical reduced/L)	37.4	2.5	40.5	3.3	35.3	1.8	34.0	2.3
[b]Total phenolics (mg/L gallic acid)	2079.0	124.0	2814.0†	141.0	2018.2	43.3	2227.1	124.8
c_{α}-Tocopherol (μmol/L)	10.9	0.8	10.0	0.4	10.0	0.4	9.2	0.4
c_{γ}-Tocopherol (μmol/L)	0.4	0.1	0.5	0.1	0.5	0.0	0.4	0.0
[c]Retinol (μmol/L)	0.7	0.0	0.7	0.0	0.6	0.0	0.6	0.0
[c]Total carotenoids (μmol/L)	0.9	0.1	0.8	0.1	0.9	0.1	0.8	0.1
[d]Ascorbic acid (mmol/L)	68.8	5.7	64.0	7.1	46.7	9.6	47.7	16.4
[e]Max diene concentration (nmol/mg LDL protein)	853.9	67.1	714.7*	53.4	859.8	7.7	867.8	130.5
[e]Max oxidation rate (nmol/mg LDL protein)	8.7	1.1	7.4	0.6	9.3	2.3	8.4	0.1
[e]Lag phase (min)	43.1	4.0	49.6	3.2	46.0	14.2	46.4	12.7
[e]TBARS (nmol/mg LDL protein)	69.1	8.3	50.5*	5.6	59.0	12.2	62.4	9.9
[f]Total cholesterol (mmol/L)	4.5	0.3	4.9	0.3	4.2	0.4	4.1	0.3
[f]Triacylglycerol (mmol/L)	0.8	0.1	0.9	0.1	0.8	0.2	0.7	0.1
[f]HDL cholesterol (mmol/L)	1.4	0.1	1.5*	0.1	1.5	0.2	1.4	0.1
[f]LDL cholesterol (mmol/L)	2.7	0.2	2.9	0.2	2.4	2.4	0.3	

Mean values were significantly different from baseline: *p ≤ 0.05, †p ≤ 0.001 (paired *t*-test)

The table shows that moderate daily intake of red wine for two weeks significantly increased plasma total phenolics ($P<0.001$) and HDL-cholesterol concentrations ($P<0.05$), and significantly reduced the maximum concentration of conjugated dienes ($P<0.05$) and TBARS ($P<0.05$) produced. No changes in any of these parameters were observed in the control group.

The results of the present study provide further insight into the possible mechanisms of moderate red wine consumption and may in part, explain the French paradox.

Renaud, S & De Logeril, M. (*Lancet, 1993)*; Burns J, et al. (*J. Agr. Food Chem. 2000)*

This study was supported by a BBSRC-Funded Case Award and by Safeway Stores plc.

DETERMINATION OF THE ANTIOXIDANT CAPACITY OF POLYPHENOLS AND RED WINES

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Red wines have antioxidant properties due to the presence of polyphenols and hydroxycinnamates. It has been reported that these compounds are efficient scavengers of peroxynitrite ($ONOO^-$) and arterial vasodilators. The antioxidant capacity of the polyphenols (catechin, epicatechin and myricetin) and different types of red wines (Cabernet Sauvignon, Malbec and blended wine) was assessed by three assays. (a) *NADH oxidation by peroxynitrite*: The $ONOO^-$ scavenging activity was higher for myricetin (IC_{50}=35 μM) than for catechin (IC_{50}=275 μM) and epicatechin (IC_{50}=313 μM). (b) *Peroxynitrite-initiated chemiluminescence in rat liver homogenate*: epicatechin (IC_{50}=7.0 μM) and catechin (IC_{50}=13 μM) were more potent than myricetin (IC_{50}=20 μM) in inhibiting the peroxynitrite-initiated chemiluminescence. (c) *Lucigenin-induced aortic rings chemiluminescence*: epicatechin (IC_{50}=15 μM) and catechin (IC_{50}=18 μM) showed higher antioxidant capacity than myricetin (IC_{50}=32 μM). All the assayed *red wines* were able to scavenge the free radical oxidant species that generate the signal in each assay. Cabernet Sauvignon was the red wine with the highest antioxidant capacity in comparison with Malbec and blended wine. Taking into account the different methods used to evaluate the antioxidant capacity of wines, we conclude that the use of sensitive biological systems (as the aorta rings) provide important information, complementing the results of chemical (NADH oxidation) and biochemical (homogenate chemiluminescence) assays, and advances in the physiological significance of antioxidants.

Supported by Fondo Vitivinícola Mendoza, Mendoza, Argentina.

DETERMINATION OF *TRANS* - RESVERATROL IN BRAZILIAN WINES

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Brazilian Vitiviniculture is concentrated mostly in Rio Grande do Sul state where the wine elaboration and the vine culture have considerable socioeconomic influence. Studies showed that after moderate wine consumption, grape antioxidant molecules could be found in blood plasma. Among them is *trans*-resveratrol (*trans*-3,5,4'-trihidroxystilbene), produced by the grapevine in response to the aggression of a fungus called *Botrytis cinerea*. Resveratrol protects from cardiovascular diseases by inhibiting the oxidation of low density lipoprotein (LDL) or bad cholesterol. *trans*-Resveratrol content was determined in commercial varieties *Vitis vinífera* wines by high performance liquid chromatography (HPLC). The samples were percolated in cellulose membranes (0.2 µm) and were injected directly in HPLC; solvents: water and acetonitrile in a flow of 1 mL.min-1; pré-column: Lichrospher 100 RP-18; column: Lichrocart C_{18}; fluorescence detector (Ex 320nm and Em 380nm). The concentration of *trans*-resveratrol presented an average of 2.14 mg.L-1 in wines of the Merlot variety, 3.00 mg.L-1 in Cabernet Sauvignon and 3.7 mg.L-1 in Tannat. Significant concentrations of *trans*-Resveratrol were found in the analyzed Brazilian wines, which varied according to several factors, including the wine variety, vinification process and the climatic conditions of the vineyard's location.

PLATELET AND HAEMOSTATIC CHANGES AFTER A MEDITERRANEAN-LIKE DIET COMPLEMENTED WITH RED WINE.

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The cardioprotective effect of the Mediterranean diet can be explained by its combined anti-atherogenic and antithrombotic effects on the plasma lipid composition and the membrane lipid bilayer of platelets and endothelial cells. We studied the antithrombotic effect of a Mediterranean-like diet with and without red wine on haemostatic factors and on blood platelet function. In a cross-over study, 10 men and 10 women, aged 25 to 59 years, on no medication, consumed a Mediterranean-like diet for 6 weeks with and 6 weeks without red wine (250 ml/day for men and 180 ml/day for women). During the experimental periods the subjects increased their intake of vegetables, cereals, fruit, mono-unsaturated fatty acids and fish at the expense of red meats and dairy products. Dietary control was by 3-day dietary records, 4 times during the study. Fasting blood samples were taken at the start (baseline), after the diet and after the diet plus wine periods. The following tests were performed: Platelet aggregation with collagen and epinephrine; membrane expression of the CD62P platelet activation marker; platelet membrane fluidity and fatty acid composition; concentrations of FVII, FVIII and fibrinogen. Platelet aggregation with collagen was unaffected, but increased significantly with epinephrine (1.1 mmol) after the diet with wine. Platelet reactivity expressed as collagen lag time was significantly prolonged after both diet periods (collagen 2 µg/ml) and after diet with wine (collagen 1 µg/ml). Platelet surface expression of CD62P was unaltered; fibrinogen and FVIII concentrations decreased, but not significantly after each intervention. FVII concentration was significantly reduced after the diet with wine period. There are some significant in vivo antithrombotic effects detectable after 6 weeks on a Mediterranean-like diet that are enhanced by moderate wine consumption.

THE LIPID PEROXIDATION STATUS OF LDL AFTER A MEDITERRANEAN-LIKE DIET WITH AND WITHOUT RED WINE

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Lipid peroxidation is regarded as an important pro-atherogenic process. This study examined whether a Mediterranean-like diet and supplementation with red wine had an impact on the conjugated dienes (CD), lipid hydroperoxides (LOOH) and thiobarbituric acid reactive substances (TBARS) status of LDL in healthy subjects who did not have severe dyslipidaemia. Subjects (N=18) increased their intake of vegetables, cereals, fruit, monounsaturated oils and fish at the expense of red meats and dairy products. After baseline studies, subjects were randomised to consumption of red wine (males 250ml and females 175ml/day) or alcohol abstinence. Fasting plasma triglycerides (TG), total cholesterol (TC), HDLC, LDLC and LDL particle size were measured. LDL was rapidly isolated and TBARS and proteins were measured before lipid extraction for CD and LOOH. 18 adults were analysed. The baseline plasma lipids were: TG 1.2 ±0.5, TC 5.3 ± 1.0, HDLC 1.3 ± 0.5 and LDLC 3.3 ± 1.0mmol/L, and LDL proteins 1.5 ± 0.4 g/L. These values did not change significantly during the study (ANOVA, $P > 0.10$) nor did LDL size. The LDL CD for baseline, –wine and +wine were 64 ± 12, 74 ± 22, 70 ± 16; LOOH were 13 ± 12, 7± 4, 8 ± 3; TBARS (- BHT) 242 ± 41, 190 ± 118, 143 ± 108 and TBARS (+ BHT) 3 ± 1, 7 ± 4, 6 ± 5 nmol/mg LDL protein respectively. The changes in LOOH and TBARS (- and + BHT) were significant (ANOVA, P= 0.014, 0.039, 0.002 respectively) but post-tests excluded an effect due to wine. The Mediterranean-like diet influenced the lipid peroxidation of LDL variably depending on the product measured whereas wine did not.

THE INFLUENCE OF A MEDITERRANEAN-LIKE DIET COMPLEMENTED WITH RED WINE ON THE CRITERIA RELATED TO THE METABOLIC SYNDROME

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The metabolic syndrome is a cluster of cardiovascular risk factors linked to insulin resistance. This study examined whether a Mediterranean-like diet complemented with red wine had an impact on the criteria related to the metabolic syndrome. In a cross over study, 10 men and 10 women between the ages 25 to 59 years, without severe dyslipidaemia and on no medication, consumed a Mediterranean-like diet for 6 weeks with and 6 weeks without red wine (250 ml for men, and 180 ml for women). During the experimental periods the subjects increased their intake of vegetables, cereals, fruit, mono-unsaturated fatty acids and fish at the expense of red meats and

dairy products. Dietary control was through 4 times 3-day dietary record during the study period. Fasting blood samples were taken at baseline, after the diet and after the diet plus wine periods. Total cholesterol (TC), triglycerides (TG), HDL cholesterol, LDL cholesterol, and LDL particle size, insulin, glucose, uric acid, BMI and blood pressure were measured. The serum lipids, LDL particle size, uric acid and insulin did not change significantly during the study compared to baseline values. Blood pressure also remained unchanged. The changes in the BMI and fasting glucose were significant compared to the baseline values. The Mediterranean-like diet had a protective effect against the metabolic syndrome, and more specifically on the impaired glucose regulation by significantly lowering the fasting glucose level after the diet plus wine period. This indicates that lifestyle changes can significantly influence the impaired response to the physiological effects of insulin including those on glucose and lipid metabolism.

ANTHOCYANINS OF *ARISTOTELIA CHILENSIS*. STRUCTURE AND POTENTIAL ANTIATHEROGENIC PROPERTIES.

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Anthocyanins are the water soluble flavonoids responsible for the red color of red wine and berries. After a preliminary screening for antioxidant capacity of berry juices, we selected the endemic Chilean berry "maqui" (*Aristotelia chilensis*) for its high antioxidant capacity (TAR and TRAP) when compared with other berrry juices. We found that maqui juice and its anthocyanin-rich fraction are effective in inhibiting two key events in atherosclerosis development. First, maqui protects LDL from oxidation in vitro and second, in human endothelial cell cultures, the addition of maqui significantly protects from hydrogen peroxide-induced intracellular oxidative stress. The anthocyanin fraction of maqui contains 83 % of total phenols and shows similar properties to the total juice. This fraction was analyzed by HPLC and 8 anthocyanins were found and then isolated by sephadex LH-20 column and preparative-HPLC. Anthocyanins separated by chromatographic methods were characterized by their UV spectra, analysis of the partial acid hydrolysis products and retention times compared to standards in HPLC. In some cases it was necessary to perform ^{1}H-NMR and ^{13}C-NMR spectra to determine the exact structure. According to these analysis the anthocyanins of maqui are delphinidins and cyanidins linked to glucose, generally in position 3 or/and 5 of the phenolic molecule. This polyglycosylation of maqui anthocyanins apparently has not been found for other berries, where generally monoglycosylated derivates have been described. These results show that maqui is a rich source of polyglycosylated anthocyanins which are effective antioxidants and suggest that maqui may have antiatherogenic properties.
(PUC-PBMEC2001)

EFFECT OF FLAVONOIDS ON THE GLUCOSE TRANSPORT OF RAT ADIPOCYTES.

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The facilitative glucose transporters (GLUTs) are integral membrane proteins responsible for the passage of hexoses through the plasma membrane. One of these transporters is GLUT4, which is expressed in muscle and fat tissue. The translocation of GLUT4 towards the cytoplasmic membrane is regulated by insulin signaling. There are clinical disorders related to abdominal fat accumulation, in which an alteration in the insulin signaling generates a loss in the translocation of transporter GLUT4 and therefore a diminution of the glucose metabolism. In the present work the effect of flavonoids on the functional activity of GLUT4 in epididymal rat adipocytes is described. Flavonoids are phenolic compounds present in fruits, vegetables and wine. The results show that the flavonoids quercetin, myricetin and catechingallate, found in the µM range in plasma, competitively inhibit the incorporation of methylglucose with a Ki of 17, 37 and 89 µM, respectively. A molecular model of the GLUT4 tridimensional structure was derived by homology. In this model we studied the effects of the flavonoids quercetin, myricetin and catechingallate on the dynamics of glucose transport. The results suggest that these flavonoids affect the activity of GLUT4 transporter by interacting with it and not by inhibiting tyrosine kinase proteins, involved in the cascade of insulin signaling.
(Support:PUC-PBMEC-2000).

INCORPORATION OF THE FLAVONOID QUERCETIN TO ERYTHROCYTES WOULD BE MEDIATED BY THE GLUCOSE TRANSPORTER GLUT1

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The facilitative glucose transporters (GLUTs) are a membrane protein family responsible for the transport of hexoses and ascorbic acid in their oxidized form (dehydroascorbic acid). In the recent years diverse biological effects of a natural compound group, the flavonoids, have been described. Flavonoids are in high concentration in fruits, vegetables and red wine. Their capacity to reduce the oxidation of LDL has been reported, as well as a beneficial effect on cardiovascular diseases and cancer. Flavonoids have the capacity to inhibit glucose and dehydroascorbic acid uptake in a competitive manner and to competitively displace cytochalasin B, a specific inhibitor that reversibly binds to GLUTs.
Using an *in Silico* 3D model of the GLUT1 transporter we studied the binding sites of glucose, dehydroascorbic acid and the flavonoid quercetin. This information was used to perform molecular dynamics to simulate the transport of these different substrates. Taking into account the results from the *in Silico* model, as well as the transport kinetics experimental data, we propose that the flavonoid quercetin enters erythrocytes by the same mechanism because it competes with the substrates glucose, dehydroascorbic acid and cytochalasin B for the same site of the GLUT1 tridimensional model. The transport of quercetin to red cells presents a Kt:0.6 mM and a Vmax: 2.75 µmoles uptake/min/10^6 cells.
(PUC-PBMEC-2000).