British Journal of Nutrition (2001), **86**, 189–195
DOI: 10.1079/BJN2001369

Red wine raises plasma HDL and preserves long-chain polyunsaturated fatty acids in rat kidney and erythrocytes

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(Received 22 March 2000 – Revised 23 January 2001 – Accepted 13 February 2001)

The effects of red wine and ethanol on plasma lipoproteins and the fatty acid composition of kidney lipids and erythrocytes phospholipids were studied. Lipid peroxidation is one of the main deleterious effects of oxidant attack on biomolecules, due to the disruption of the structural integrity of membranes. The vulnerability of the kidney to oxidative damage has been partly attributed to its high content of long-chain polyunsaturated fatty acids. Antioxidants, such as flavonoids, would be a means of reducing the risk of oxidative damage to membranes. Nutritional sources rich in antioxidants, including those provided by wine, are expected to attenuate the effects of oxidative challenges. Adult rats were fed red wine rich in flavonols, ethanol (125 ml/l), or alcohol-free red wine. The control group drank water. After 10 weeks, blood samples served to measure plasma lipoproteins and antioxidant capacity. Kidney lipids and erythrocyte phospholipids were extracted. The samples were assayed by GLC. Energy intake did not differ between all the groups, but the weight gain of the ethanol group was less than the other three groups. Blood HDL and triacylglycerols were increased by both ethanol and red wine. Ethanol decreased arachidonic and docosahexaenoic acids in both kidney lipids and erythrocyte phospholipids, as compared with either water, red wine or alcohol-free red wine groups. These results indicate that non-alcoholic components of red wine could contribute to avoiding the unfavourable effects of ethanol on plasma lipoproteins, kidney lipids and membrane erythrocyte phospholipids.

Red wine: Kidney lipids: Erythrocyte phospholipids

A considerable body of evidence has implicated reactive oxygen species in renal damage caused by various mechanisms, including ischaemia, toxaemia, or immunological diseases (Baliga *et al.* 1997; Fryer, 1997). One of the main deleterious effects of reactive oxygen species is the peroxidation of lipids, and the disruption of the structural integrity of membranes, thus altering the capacity for cell transport processes (Baud & Ardaillou, 1993). In addition, it was argued that peroxidation of lipoproteins could play a key role in the progression of renal failure (Gröne *et al.* 1994). In addition, it has been reported that hyperlipoproteinaemia can aggravate glomerulosclerosis and chronic tubulointerstitial damage, due to increased generation of reactive oxygen species (Fiorillo *et al.* 1998; Scheuer *et al.* 2000). In contrast, renal damage caused by hypercholesterolaemia and oxidation of LDL was attenuated by antioxidants such as ascorbic acid (Lee *et al.* 1997). It was demonstrated that red wine polyphenols reduce the susceptibility of LDL to oxidation *in vivo* (Nigdikar *et al.* 1998). In addition, HDL, particularly abundant in the plasma of the rat, also inhibits the oxidation of LDL (Parthasarathy *et al.* 1990). The high vulnerability of kidney to lipid peroxidation has been partly attributed to its high content of long-chain polyunsaturated fatty acids (PUFA), such as arachidonic (ARA) and docosahexaenoic (DHA) acids (Kubo *et al.* 1997). On the other hand, increased microsomal and peroxisomal fatty acid oxidation by rat kidney following chronic ethanol consumption has been found (Orellana *et al.* 1998). Nevertheless, the involvement of this finding in the pathogenesis of the renal damage induced by ethanol has not been established (Rodrigo *et al.* 1998). In addition, it was found that

Abbreviations: ARA, arachidonic acid; DHA, docosahexaenoic acid; FRAP, ferric-reducing ability of plasma; PUFA, polyunsaturated fatty acids; TG, triacylglycerol.

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erythrocytes of patients with chronic renal failure showed increased lipid peroxidation associated with a reduction in long-chain PUFA (Peuchant *et al.* 1997). Antioxidants, such as flavonoids, would be a means of diminishing the risk to both kidney and erythrocytes of oxidative damage caused by reactive oxygen species, due to their biological activities ascribed to radical scavenging, metal chelating and enzyme modulation ability (Shimoi *et al.* 1997; Pietta *et al.* 1998). Moreover, wine flavonoids, such as quercetin, may exert a protective effect against cytotoxicity of reactive oxygen species due to their membrane affinity (Kuhlman *et al.* 1998). It was found that resveratrol, a hydroxystilbene related to flavonoids, inhibits LDL oxidation due to its chelating ability (Frémont *et al.* 1999). Therefore, nutritional sources rich in antioxidants, including those provided by wine, are expected to attenuate the damage caused by oxidative challenges. Although the mechanism of this effect remains unclear, the polyphenolic compounds of wine, are particularly abundant in Chilean red wine (McDonald *et al.* 1998), and could reinforce the antioxidant system responsible for counteracting the effects of reactive oxygen species. In fact, an increased plasma antioxidant capacity was found in human subjects after ingestion of moderate amounts of both red wine (Duthie *et al.* 1998; Durak *et al.* 1999) or alcohol-free red wine (Serafini *et al.* 1998). It was also found recently that moderate consumption of red wine protects the rat against oxidation *in vivo* (Roig *et al.* 1999). However, the relative contribution of ethanol or the non-alcoholic components of red wine to these protective effects remains to be elucidated. The aim of the present study was to determine the effect of moderate consumption of either ethanol or red wine rich in flavonols on plasma levels of HDL and the long-chain PUFA composition of kidney and erythrocytes of the rat.

Materials and methods

Animals and diet

The study protocol was approved by the Comité de Bioética, Programa de Farmacología Molecular y Clínica, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile. Eighty male Wistar rats (Departamento de Nutrición, Facultad de Medicina, Universidad de Chile), weighing 200 (SE 6) g, were randomly assigned to one of the following four groups, each allowing free access to food (experimental diet). The animals were given, as sole drinking fluid, the following beverages. Group 1: red wine (Cabernet Sauvignon, 1998 harvest, Viña Lomas de Cauquenes, Cauquenes Valley, Chile); group 2: alcohol-free red wine (made from the same red wine, from which the alcohol had been removed by evaporation at 25°C for 4 h. To avoid mechanical stress, the vacuum was applied progressively and gradually up to −3 MPa (Serafini *et al.* 1998), group 3: water (tap water); group 4: ethanol (aqueous ethanol solution having the same ethanol concentration of the red wine used for group 1 (125 ml/l). The composition of the experimental diet is shown in Table 1. Daily fluid intake was measured with graduated Richter tubes. Food intake was also estimated by gravimetry. Blood

Table 1. Composition of the experimental diet (g/kg diet)

Casein	200
DL-methionine	3
Corn starch	297
Sucrose	225
Canola oil	100
Potato starch	25
Water-soluble vitamins*	30
Fat-soluble vitamins†	20
Mineral mixture‡	50
Fibre	50
Energy (MJ/kg)	16·3‖

* The water-soluble vitamin composition was (g/kg diet): choline chloride 0·945, *p*-aminobenzoic acid 0·473, inositol 0·094, niacin 0·047, calcium pantothenate 0·024, riboflavin 0·024, thiamine hydrochloride 0·019, pyridoxine hydrochloride 0·005, folic acid 0·005, biotin 0·001, cyanocobalamin 0·0005.

† The fat-soluble vitamins composition was (per kg diet): DL-α-tocopherol acetate 66 mg, all-*trans*-retinyl acetate 2·5 mg (7000 IU), menadione 60 μg, cholecalciferol 7·5 μg.

‡ The mineral composition was (per kg diet): $CaCO_3$ 15·75 g, $CaH_2PO_4.2H_2O$ 3·35 g, K_2HPO_4 16·40 g, NaCl 8·52 g, $MgSO_4.7H_2O$ 5·18 g, iron citrate 0·51 g, $MnSO_4.H_2O$ 0·25 g, $CuSO_4.5\ H_2O$ 25·3 mg, $ZnCl_2$ 5·0 mg, KI 1·2 mg, sodium selenite 5·0 mg, NaF 5·0 mg.

‖ 3900 kcal/kg.

samples from each group were obtained through the carotid artery after anaesthesia using 20 % urethane at a dose of 2 g/kg body weight. The samples were received into plastic tubes with EDTA and centrifuged immediately to separate erythrocytes from plasma. The kidneys were perfused with buffer (0·01 M-Tris, pH 7·40). Plasma and tissues samples were stored at −70°C until the analyses were performed. Blood ethanol levels were determined in red wine and ethanol groups by an enzymic micromethod (Brink *et al.* 1954). The antioxidant capacity of plasma was measured by the method of Benzie & Strain (1996), based on the ferric reducing ability of plasma (FRAP), expressed as μM. The red wine was chosen out of twenty-five samples of Cabernet Sauvignon red wine, based on a recent report showing that Chilean red wines contain higher concentrations of flavonols than their counterparts from different geographical regions (McDonald *et al.* 1998). Cabernet Sauvignon grapes have a high skin:volume ratio which appears to be associated with the production of flavonol-rich wines. The quantitative analysis of samples was performed by reversed-phase HPLC (Croizier *et al.* 1997). The concentration of flavonols was assessed by measuring the content of free and conjugated myricetin and quercetin, the major representatives of the flavonol subclass, which could reflect the content of total polyphenols of red wine.

Fatty acid analysis of kidney

Total lipids were extracted from kidney homogenates with methanol – chloroform (2:1, v/v). The chloroform layer was dried under N_2, methylated and the fatty acid methyl esters were extracted with hexane prior to capilllary GLC analysis.

Fatty acid analysis of erythrocytes

Erythrocytes membranes were separated (Steck *et al.* 1970; Huertas *et al.* 1999), total lipids were extracted (Bligh &

Dyer, 1959) and phospholipids were fractionated by TLC (Silica gel 60) with a solvent system of chloroform – methanol – acetic acid – Water (50:37·5:3·5:2, by vol.). Afterwards, the lipid spots on the chromatograms were scraped, methylated and the fatty acid composition of phospholipid fractions was analysed using a GC model 6890; Hewlett Packard (Palo Alto, CA, USA), equipped with apolar capillary column (BPX × 70, USGE, length 50 m, diameter 0·22 mm). The oven temperature was programmed from 180 to 230°C, at 2°C/min, with a final hold, to separate the fatty acids from 14:0 to 22:6*n*-3. The temperature of both detector and injector was 240°C. H was used as carrier gas (flow rate 1·5 ml/min) and a split of 1:80. The fatty acid methyl esters were identified by comparison with authentic standards retention times and peak areas were automatically computed as a percentage by a Hewlett Packard HP 3396 series III integrator. Identification of the individual methyl esters was performed by frequent comparisons with authentic standard mixtures analysed under the same conditions.

Analysis of lipids and lipoproteins

Total cholesterol, triacylglycerol (TG) and HDL-cholesterol, were determined in plasma by an enzymic assay (Boehringer-Mannheim, Roche Diagnostics GmbH. D-68298, Mannheim, Germany). LDL-Cholesterol was calculated from the formula: LDL-cholesterol (mg/l) = (total cholesterol − T6/5 − HDL)×10.

Chemicals

The reagents were purchased from Sigma-Aldrich (St Louis, MO, USA), Merck (Darmstadt, Germany) and Riedel-de Haën (Germany), and were of the highest commercial grade available. Casein was purchased from B. Braun Medical SA (Santiago, Chile). Rat pellets were from Champion SA (Santiago, Chile).

Statistical analyses

Results are expressed as means with their standard errors. All statistical analyses of data were computed using Statistical Analysis System (SAS Institute Inc., Cary NC, USA). The results for each specific fatty acid and different plasma lipoproteins were assessed by ANOVA, and the comparisons for individual differences between groups were done using the Scheffé test. The differences were considered statistically significant at $P < 0·05$.

Results

Analysis of red wine, blood ethanol levels and plasma antioxidant power

Total flavonols (free and conjugated myricetin and quercetin) reached values of 55·2 (SE 2·3) mg/l, with a quercetin:myricetin ratio 0·8. Free fraction of total flavonols was 44·2 (SE 1·8) %. Blood ethanol levels for the red wine and ethanol groups and plasma antioxidant power, assessed by FRAP, for each group are given in Table 2. Blood ethanol levels of the red wine and ethanol groups were not significantly different from each other, which is related to the similar fluid intakes of both groups (79·8 (SE 12·9) *v.* 71·5 (SE 6·3) ml/d per kg body weight, for wine *v.* ethanol respectively). Red wine and alcohol-free red wine consumption resulted in increased values of FRAP, compared with the water group, the increase being more marked in the case of the red wine group ($P < 0·05$).

Energy consumption and body-weight gain

The daily energy intake during the experimental feeding period (70 d) was the same for the four groups: 879 (SE 75) kJ/d per kg body weight (210 (SE 18) kcal/d per kg body weight). The contributions of ethanol consumption to energy intakes for the ethanol and red wine groups were 24 % and 28 %, respectively, but were not significantly different. Fluid intake was similar for all groups (mean 85 ml/d per kg body weight). Body-weight gain (g/d per kg body weight) in the alcohol-free red wine group was 31·7 (SE 3·0) and it was significantly higher than the average of the weight gain of every other group: 21·7 (SE 4·0) ($P < 0·05$).

Plasma levels of cholesterol, lipoproteins and triacylglycerol

Plasma levels of lipoproteins and TG of the four experimental groups are presented in Table 3. Total cholesterol was not modified by the different treatment. Levels of HDL of the ethanol, red wine and alcohol-free red wine groups were significantly higher than the water group ($P < 0·05$). Plasma TG of the ethanol and red wine

Table 2. Blood ethanol levels (mg/l) and plasma antioxidant capacity (μM) for the experimental groups‡
(Mean values with their standard errors for twenty rats per group)

	Groups							
	Ethanol		Water		Red wine		Alcohol-free red wine	
Variables	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Blood ethanol	343·3	15·6	–		570·3	82·0	–	
FRAP	254·4	20·5	259·2	13·5	364·1*	18·9	295·7*†	21·9

FRAP, ferric-reducing ability of plasma.
* Mean values were significantly different from the water group: (*$P < 0·05$) (ANOVA and Scheffé tests).
† Mean value was significantly different from the red wine group: (†$P < 0·05$) (ANOVA and Scheffé tests).
‡ For details of diets and procedures, see Table 1 and p. 190.

Table 3. Plasma levels of cholesterol, lipoproteins and triacylglycerol for the experimental groups (mg/l)*
(Mean values with their standard errors for twenty rats per group)

	Groups							
	Ethanol		Water		Red wine		Alcohol-free red wine	
Lipid	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Total Chol	911	82	906	66	948	89	926	39
HDL-Chol	438[a]	14	340[b]	20	429[a]	27	509[a]	51
LDL-Chol	229	3	237	23	265	22	212	15
Triacylglycerol	1120[a]	141	718[b]	134	1400[a]	102	810[b]	86
HDL:LDL	21[a]	4	14[a]	1	20[a]	2	27[b]	2

Chol, cholesterol.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0{\cdot}05$).
* For details of diets and procedures, see Table 1 and p. 190.

groups was significantly higher than either water or alcohol-free red wine groups ($P < 0{\cdot}05$).

Fatty acid composition of kidney lipids

The composition of fatty acids in kidney lipids for the experimental groups are shown in Table 4. ARA (20:4*n*-6) and eicosapentaenoic acid (20:5*n*-3), and total long-chain PUFA in the ethanol group were significantly ($P < 0{\cdot}05$) lower than those of any other group. In addition, 20:4*n*-6:18:2*n*-6 was reduced in ethanol group and increased in alcohol-free red wine group, as compared with the water group.

Fatty acid composition of membrane erythrocyte phospholipids

Table 5 shows the contents of fatty acids of membrane erythrocytes of the experimental groups. Long-chain PUFA in the ethanol group were significantly lower than those of the water group, whereas the values for the red wine and alcohol-free red wine groups were not different from those of the water group ($P < 0{\cdot}05$). The ethanol and red wine groups showed diminished levels of ARA and 22:6*n*-3 as compared with the water group. DHA was diminished by ethanol, red wine and alcohol-free red wine ($P < 0{\cdot}05$). The 20:4*n*-6:18:2*n*-6 and 22:6*n*-3:18:3*n*-3 ratios were significantly reduced in ethanol group, whereas the 22:6*n*-3:18:3*n*-3 ratio was significantly increased in the red wine and alcohol-free red wine groups ($P < 0{\cdot}05$) as compared with the water group.

Discussion

The purpose of the present study was to investigate the effects of ethanol and wine rich in flavonols on plasma HDL, LDL and TG and the fatty acid composition of kidney lipids and erythrocytes phospholipids. Our data provide evidence for increased TG and HDL without changes to total cholesterol and LDL after 10 weeks of treatment either with red wine or an equivalent concentration of alcohol as aqueous ethanol. Ethanol consumption is

Table 4. Fatty acid composition of kidney lipids (g/100 g methyl ester) for the experimental groups*
(Mean values with standard errors for twenty rats per group)

	Groups							
	Ethanol		Water		Red wine		Alcohol-free red wine	
Fatty acid	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Saturated†	40	2·7	38·5	1·5	36	0·3	36·5	0·6
Monounsaturated‡	0·6	0·4	11·7	1·2	11·6	0·3	11·7	0·5
18:2*n*-6	2·5	0·2	2·3	0·2	2·2	0·3	2·2	0·2
20:4*n*-6	21·6[a]	1·4	28·6[b]	0·3	28·3[b]	0·7	30·4[b]	0·1
18:3*n*-3	0·35[a]	0·01	0·42	0·01	0·41	0·01	0·4	0·02
20:5*n*-3	0·71[a]	0·06	2·0[b]	0·3	1·55[b]	0·2	1·42[b]	0·01
22:6*n*-3	0·51[a]	0·2	0·9[b]	0·2	0·53[a]	0·1	0·7[b]	0·2
Total *n*-6	37·2[a]	1·8	32·7[b]	0·3	47·6[c]	0·6	47·6[c]	0·5
Total *n*-3	3·2[a]	0·1	6·4[b]	0·5	6·6[b]	0·2	5·2[b]	0·2
Total long-chain PUFA§	24·3[a]	0·1	33·4[b]	0·3	31·7[b]	0·7	34·7[b]	0·2
20:4*n*-6 : 18:2*n*-6	8·69[b]	0·5	12·71[a]	0·9	13·24[a]	0·8	14·75[c]	0·1
20:5*n*-3 : 18:3*n*-3	2·65[b]	0·3	5·76[a]	0·3	4·43[b]	0·2	7·71[a]	1·3

PUFA, polyunsaturated, fatty acids.
[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P < 0{\cdot}05$).
* For details of diets and procedures, see Table 1 and p. 190.
† Saturated: 14:0, 16:0, 18:0, 20:0, 22:0.
‡ Monounsaturated: 16:1*n*-7, 18:1*n*-9.
§ Total long-chain PUFA: $\geq C_{20}$.

Table 5. Fatty acid composition (g/100 g methyl esters) of membrane phospholipids of erythrocytes for the experimental groups*
(Mean values with standard errors for twenty rats per group)

	Groups							
	Ethanol		Water		Red wine		Alcohol-free red wine	
Fatty acid	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Saturated†	33·1	1·29	32·6	0·6	29·2	0·7	32·6	0·4
Monounsaturated‡	26·1[a]	1·0	14·1[b]	1·0	16·9[b]	0·5	13·8[b]	0·8
18:2*n*-6	2·7	0·1	2·9	0·4	2·9	0·4	2·6	0·1
20:4*n*-6	18·5[a]	0·4	23·1[b]	0·4	24·9[b]	0·9	22·6[b]	0·01
18:3*n*-3	0·51[a]	0·1	1·54[b]	0·2	3·4[c]	0·01	0·8[a]	0·01
20:5*n*-3	2·2[a]	0·1	1·8[b]	0·01	1·2[b]	0·8	2·2[a]	0·1
22:6*n*-3	3·6[a]	0·4	6·2[b]	0·2	4·3[ac]	0·3	4·9[c]	0·3
Total *n*-6	36·9	0·6	35·8	1·0	30·5	1·2	38	0·4
Total *n*-3	6·3[a]	0·4	9·6[b]	0·3	8·9[b]	0·2	7·8[b]	0·4
Total PUFA	42·8[a]	0·9	38·7[b]	1·1	31·3[b]	1·2	45·1[a]	0·5
Total long-chain PUFA§	40·1[a]	0·4	43·8[b]	0·2	41·8[b]	0·1	43·3[b]	0·5
20:4*n*-6 : 18:2*n*-6	6·85[b]	0·5	8·21[a]	1·9	8·71[a]	2·4	8·62[a]	0·8
22:6*n*-3 : 18:3*n*-3	1·26[a]	0·1	4·10[b]	0·5	6·80[c]	0·8	7·79[c]	0·2

PUFA, polyunsaturated fatty acids.
[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
* For details of diets and procedures, see Table 1 and p. 190.
† Saturated: 14:0, 16:0, 18:0, 20:0, 22:0.
‡ Monounsaturated: 16:1*n*-7; 18:1*n*-9.
§ Total long-chain PUFA $\geq C_{20}$.

one of the most frequent causes of increasing plasma TG (Ayaori *et al.* 1997) and may affect a number of steps in plasma lipoprotein metabolism. In fact, ethanol serves as substrate for lipoprotein and TG synthesis. In addition, key enzymes involved in lipoprotein metabolism, such as lipoprotein lipase, hepatic lipase, and cholesterol ester transfer protein are affected by ethanol (Fröhlich, 1996). Pownall *et al.* (1999) reported that alcohol increases plasma levels of TG in normolipidaemic subjects, but this effect is less marked in the presence of hypertriacylglycerolaemia.

The ability of ethanol to increase plasma levels of HDL, the most abundant plasma lipoprotein in the rat has been well documented (Branchi *et al.* 1997). These data are in agreement with the results reported here (Table 3), showing that plasma HDL of both the ethanol and red wine groups are significantly ($P < 0.05$) higher than that of the water group. Since the HDL of rats treated with alcohol-free red wine was also higher than that of the water group, mechanisms other than those exerted by ethanol may operate. It was reported that red wine inhibits the cell-mediated oxidation of lipoproteins, but white wine is less effective (Rifici *et al.* 1999). Therefore, it seems likely that non-alcoholic wine components, such as the particularly abundant polyphenols of red wine, may also play a role to explain the effect of wine on lipoprotein metabolism. At least two hypotheses could be put forward to explain the augmentation of HDL. First, red wine polyphenols may cause a stabilization of plasma lipoproteins, partly due to increased levels of FRAP. Second the systemic effect of wine polyphenols, known to modulate various enzyme activities, could have an effect on lipoprotein metabolism leading to an augmentation of HDL. However, further studies about the metabolic effects of these compounds are still lacking to support this view.

The red wine used in the present investigation had the highest content of total flavonols (55·2 mg/l) of all available results in the literature, according to data from McDonald *et al.* (1998). The authors reported values ranging from 4·6 to 41·6 mg/l, after analysing sixty-five samples of red wines from a wide range of geographical origins. The augmentation of antioxidant capacity of plasma following red wine consumption (Table 2) is in agreement with data found in human subjects (Durak *et al.* 1999). Although it could be questioned whether the FRAP is equivalent to an antioxidant effect, the significantly increased FRAP levels found in the red wine and ethanol-free red wine groups could be at least partly attributed to the absorbed polyphenols. Moreover, absorbed quercetin, a strong wine antioxidant that prevents oxidation of LDL *in vitro*, is metabolized to conjugated derivatives retaining antioxidant properties in plasma (Manach *et al.* 1998). The bioavailability of wine polyphenols remains to be fully established. However, in human subjects, it was reported that only free flavonols are able to pass through the gut wall (Hollman & Katan, 1997). Since the free fraction of flavonols contained in the red wine used in the present study reached 44·2 %, it should be expected that they contribute to enhancement of the antioxidant capacity of plasma, thereby accounting for the FRAP levels shown by red wine and free-alcohol red wine groups. It should be noted that other plasma antioxidants, such as ascorbate, protein thiols or urate, could also influence the FRAP levels; however, no significant changes in the dietary intake were found between all the experimental groups. On the other hand, the finding of a more marked increase of FRAP in the wine group, compared with the alcohol-free red wine group, could be partly explained on the basis of the wine ethanol content aiding phenolic absorption (Duthie *et al.* 1998). Nevertheless, it should not be discounted that the contribution of some unidentified volatile compounds could be lost from the red wine during the process of dealcoholization.

The effects of ethanol, red wine and alcohol-free red wine were also examined in terms of changes in fatty acid composition of kidney lipids and erythrocytes membrane phospholipids. The analysis of fatty acids in erythrocyte membrane phospholipids was undertaken to ascertain whether they reflect those of other organs such as kidney. The administration of ethanol (125 ml/l) caused a diminution of kidney long-chain PUFA (ARA and DHA), and the ARA:linoleic acid ratio (20:4*n*-6:18:2*n*-6 ratio) was significantly ($P < 0{\cdot}05$) lower than that of all the other groups. After ethanol treatment, ARA in membrane erythrocytes was significantly ($P < 0{\cdot}05$) decreased. In kidney and erythrocytes, the decrease of ARA may be due to a decreased synthesis, based on the lower 20:4*n*-6:18:2*n*-6 ratio found in this group. In addition, in kidney, ethanol could increase ARA utilization for the synthesis of eicosaenoids, as occurs in the liver (Okita *et al.* 1997). In addition, ARA and DHA in kidney could be diminished by an increased utilization via fatty acid oxidation, as supported by previous studies (Orellana *et al.* 1998). A significant finding is the lack of difference between ARA levels of the red wine and alcohol-free red wine groups, despite the presence of ethanol in the former. These results could indicate that the presence of non-alcoholic components of wine (e.g. polyphenols) abolishes the effect of ethanol on kidney and erythrocytes ARA levels. Alternatively, phospholipase A_2 was found to be activated by ethanol in membrane fractions of brain, as well as in a variety of organs (Hungund *et al.* 1994). Although no studies of the effect of ethanol on this enzyme have been reported in kidney, a similar response would explain the ARA diminution, since phospholipase A_2 shows preference for ARA at the *sn*-2 position of 1,2-*sn*-diacylglycerol. (Basaravajappa *et al.* 1999).

Unchanged long-chain PUFA contents of kidney and erythrocytes, found in the red wine group, in contrast with the diminished levels shown by the ethanol group, may be interpreted as the result of a cytoprotective effect exerted by red wine polyphenols. This hypothesis is supported by the demonstration that the major polyphenols of red wine inhibit the synthesis of eicosaenoids through mechanisms including inhibition of phospholipase A_2 and cyclooxygenase (Soleas *et al.* 1997), thereby avoiding the reduction in ARA otherwise caused by the presence of ethanol.

Our present data, together with those of the literature, lead us to suggest that the reduction of ARA, as a representative of all long-chain PUFA, could be used as a marker for the unfavourable effect of ethanol consumption on plasma lipoproteins and membrane phospholipids. Nevertheless, ARA could be unaltered, despite ethanol intake, whenever diet conditions supply the antioxidants able to counteract this impairment in lipid membranes.

Acknowledgements

This research was supported by the Fondo Nacional de Ciencia y Tecnología (FONDECYT, grant 1990784). We would like to thank to Viña Lomas de Cauquenes, Cauquenes Valley, Chile, for providing the red wine used for the studies. The technical assistance of Diego Soto and Claudio Vilches are also acknowledged.

References

Ayaori M, Ishikawa T, Yoshida H, Suzukawa M, Nishiwaki M, Shige H, Ito T, Nakajima K, Higashi K, Yonemura A & Nakamura H (1997) Beneficial effects of alcohol withdrawal on LDL particle size distribution and oxidative susceptibility in subjects with alcohol-induced hypertriglyceridemia. *Arteriosclerosis, Thrombosis, and Vascular Biology* **17**, 2540–2547.

Baliga R, Ueda N, Walker PD & Shah SV (1997) Oxidant mechanisms in toxic acute renal failure. *American Journal of Kidney Diseases* **29**, 465–477.

Basaravajappa BS, Cooper TB & Hungund BL (1999) Effect of chronic ethanol exposure on mouse brain arachidonic acid specific phospholipase A2. *Journal of Neurochemistry* **72**, 522–528.

Baud L & Ardaillou R (1993) Involvement of reactive oxygen species in kidney damage. *British Medical Bulletin* **49**, 621–629.

Benzie IFF & Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry* **239**, 70–76.

Bligh EG & Dyer WJ (1959) A rapid method of total lipid extraction. *Canadian Journal of Biochemistry and Physiology* **37**, 911–917.

Branchi A, Rovellini A, Tomella C, Sciariada L, Torri A, Molgora M & Sommariva D (1997) Association of alcohol consumption with HDL subpopulations defined by apolipoprotein A-I and apolipoprotein A-II content. *European Journal of Clinical Nutrition* **51**, 362–365.

Brink NG, Bonnichsen R & Theorell H (1954) A modified method for the enzymatic microdetermination of ethanol. *Acta Pharmacologica et Toxicologica* **10**, 223–236.

Crozier A, Jensen E, Lean MEJ & McDonald MS (1997) Quantitative analysis of flavonoids by reversed phase high performance liquid chromatography. *Journal of Chromatography* **761**, 315–321.

Durak I, Burak Cimen MY, Büyükkoçak S, Kaçmaz M & Öztürk S (1999) The effect of red wine on blood antioxidant potential. *Current Medical Research and Opinion* **15**, 208–213.

Duthie GG, Pedersen MW, Gardner PT, Morrice PC, Jenkinson AM, McPhail DB & Steele GM (1998) The effect of whisky and wine consumption on total phenol content and antioxidant capacity of plasma from healthy volunteers. *European Journal of Clinical Nutrition* **52**, 733–736.

Fiorillo C, Oliveiro C, Rizzuti G, Nediani C, Pacini A & Nassi P (1998) Oxidative stress and antioxidant defenses in renal patients receiving regular haemodialysis. *Clinical Chemistry and Laboratory Medicine* **36**, 149–153.

Frémont L, Belguendouz L & Delpal S (1999) Antioxidant activity of resveratrol and alcohol-free wine polyphenols related to LDL oxidation and polyunsaturated fatty acids. *Life Sciences* **64**, 2511–2521.

Frohlich JJ (1996) Effects of alcohol on plasma lipoprotein metabolism. *Clinica Chimica Acta* **246**, 39–49.

Fryer MJ (1997) Vitamin E may slow kidney failure owing to oxidative stress. *Redox Report* **3**, 259–261.

Gröne EF, Walli AK, Gröne HJ, Miller B & Seidel D (1994) The role of lipids in nephrosclerosis and glomerulosclerosis. *Atherosclerosis* **107**, 1–13.

Hollman PC & Katan MB (1997) Absorption, metabolism and health effects of dietary flavonoids in man. *Biomedicine & Pharmacotherapy* **51**, 305–310.

Huertas JR, Palomino N, Ochoa JJ, Quiles JL, Ramírez-Tortosa MC, Battino M, Robles R & Mataix J (1998) Lipid peroxidation and antioxidants in erythrocyte membranes of full-term and preterm newborns. *Biofactors* **8**, 133–137.

Hungund BL, Zheng Z, Lin L & Barkai AI (1994) Ganglioside

GM1 reduces ethanol induced phospholipase A2 activity in synaptosomal preparations from mice. *Neurochemistry International* **25**, 321–325.

Kubo K, Saito M, Tadokoro T & Maekawa A (1997) Changes in susceptibility of tissues to lipid peroxidation after ingestion on various levels of docohexaenoic acid and vitamin E. *British Journal of Nutrition* **78**, 655–669.

Kuhlmann MK, Horsh E, Burkhardt G, Wagner M & Kohler H (1998) Reduction of cysplatin toxicity in cultured renal tubular cells by the bioflavonoid quercetin. *Archives of Toxicology* **72**, 536–540.

Lee HS, Jeong JY, Kim BC, Kim YS, Zhang YZ & Chung HK (1997) Dietary antioxidant inhibits lipoprotein oxidation and renal injury in experimental focal segmental glomerulosclerosis. *Kidney International* **51**, 1151–1159.

McDonald MS, Hughes M, Burns J, Lean MEJ, Matthews D & Crozier A (1998) Survey of the free and conjugated myricetin and quercetin content of red wines of different geographical origins. *Journal of Agricultural and Food Chemistry* **46**, 368–375.

Manach C, Morand C, Crespy V, Demigné C, Texier O, Régérat F & Rémésy C (1998) Quercetin is recovered in human plasma as conjugated derivatives which retain antioxidant properties. *FEBS Letters* **426**, 331–336.

Nigdikar SV, Williams NR, Griffin BA & Howard AN (1998) Consumption of red wine polyphenols reduces the susceptibility of low-density lipoproteins to oxidation in vivo. *American Journal of Clinical Nutrition* **68**, 258–265.

Okita M, Suzuki K, Sasagawa T, Yamamoto J, Miyamoto A, Wakabayashi H & Watanabe A (1997) Effect of arachidonate on lipid metabolism in ethanol-treated rats fed with lard. *Journal of Nutritional Science and Vitaminology* **43**, 311–326.

Orellana M, Valdés E, Fernández J & Rodrigo R (1998) Effects of chronic ethanol consumption on extramitochondrial fatty acid oxidation and ethanol metabolism by rat kidney. *General Pharmacology* **30**, 719–723.

Parthasarathy S, Barnett J & Fong LG (1990) High-density lipoprotein inhibits the oxidative modification of low-density lipoprotein. *Biochimica et Biophysica Acta* **1044**, 275–283.

Peuchant E, Delmas-Beauvieux MC, Duborg L, Thomas MJ, Perromat A, Aparicio M, Clerc M & Combe C (1997) Antioxidant effects of a supplemented very low protein diet in chronic renal failure. *Free Radical Biology and Medicine* **22**, 313–320.

Pietta P, Simonetti P, Gardana C, Brusamolino A, Morazzoni P & Bombardelli E (1998) Relationship between rate and extent of catechin absorption and plasma oxidant status. *Biochemistry and Molecular Biology International* **46**, 895–903.

Pownall HJ, Ballantyne CM, Kimball KT, Simpson SL, Yeshurun D & Gotto AM Jr (1999) Effect of moderate consumption on hypertriglyceridemia: a study in the fasting state. *Archives of Internal Medicine* **159**, 981–987.

Rifici VA, Stephan EM, Schneider SH & Khachadurian AK (1999) Red wine inhibits the cell-mediated oxidation of LDL and HDL. *Journal of the American College of Nutrition* **18**, 137–143.

Rodrigo R, Thielemann L, Olea M, Muñoz P, Cereceda M & Orellana M (1998) Effect of ethanol ingestion on renal regulation of water and electrolytes. *Archives of Medical Research* **29**, 209–218.

Roig R, Cascón E, Arola L, Bladé C & Salvadó MJ (1999) Moderate red wine consumption protects the rat against oxidation in vivo. *Life Sciences* **64**, 1517–1524.

Scheuer H, Gwinner W, Hohbach J, Gröne EF, Brandes RP, Malle E, Olbricht CJ, Walli AK & Gröne HJ (2000) Oxidant stress in hyperlipidemia-induced renal damage. *American Journal of Physiology* **278**, F63–F74.

Serafini M, Malani G & Ferro-Luzzi A (1998) Alcohol-free red wine enhances plasma antioxidant capacity in humans. *Journal of Nutrition* **128**, 1003–1007.

Soleas GJ, Diamandis EP & Goldberg DM (1997) Wine as a biological fluid: history, production, and role in disease prevention. *Journal of Clinical Laboratory Analysis* **11**, 287–313.

Shimoi K, Shen B, Toyokuni S, Mochizuki R, Furugori M & Kinae N (1997) Protection by alpha G-rutin, a water-soluble antioxidant flavonoid, against renal damage in mice treated with ferric nitrilotriacetate. *Japanese Journal of Cancer Research* **88**, 453–460.

Steck TL, Weinstein RS, Straus JH & Wallach DF (1970) Inside-out red cell membrane vesicles: preparation and purification. *Science* **168**, 255–257.