Grape products and cardiovascular disease risk factors

Jara Pérez-Jiménez[1]* and Fulgencio Saura-Calixto[2]

[1]*Departamento de Nutrición y Bromatología I, Facultad de Farmacia, Universidad Complutense de Madrid, Ciudad Universitaria, 28040 Madrid, Spain*
[2]*Departamento de Metabolismo y Nutrición, IF-ICTAN, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain*

Many *in vivo* trials have evaluated the effects of grape products on different CVD risk factors. Most published studies have dealt with some specific aspects of mechanisms of grape flavonoid action or have focused only on one product, such as wine. The aim of the present paper is to review trials dealing with grape products and CVD published during the last 13 years (seventy-five trials). Polyphenols, alcohol and dietary fibre are the main constituents of the tested products. In animal and human studies, grape products have been shown to produce hypotensive, hypolipidaemic and anti-atherosclerotic effects, and also to improve antioxidant status as measured in terms of plasma antioxidant capacity, oxidation biomarkers, antioxidant compounds or antioxidant enzymes. Differences in the design of the studies and in the composition of the tested products (not always provided) could explain the different results of these studies.

Grapes: Wine: Grape products: Cardiovascular disease

Introduction

CVD continues to be a leading cause of morbidity and mortality among adults in Western countries. Cigarette smoking, high blood pressure, high serum total cholesterol and LDL-cholesterol, low serum HDL-cholesterol, diabetes mellitus and advanced age are considered the main risk factors for CVD[1,2].

A large number of epidemiological studies have associated a diet rich in fruits and vegetables with a reduction in CVD risk factors[3]. This is presumably due to the presence in plant foods and certain beverages of a variety of compounds including different kinds of antioxidants, such as vitamin C, vitamin E, polyphenols and carotenoids. Polyphenols in particular have been associated with a reduction of the risk of different diseases in several epidemiological studies[4].

The French paradox[5], i.e. the low prevalence of CVD in certain French regions with a high intake of saturated fats, has been put down to the consumption of red wine. This effect has been attributed mainly to the presence of polyphenols, a large group of compounds present in plant foods and beverages that have demonstrated a strong *in vitro* antioxidant capacity due to their ability to scavenge free radicals and to chelate metals[6]. Ethanol can also improve the bioavailability of polyphenols, as well as playing a specific cardioprotective role.

The possible benefits of grape and wine consumption in relation to CVD have prompted researchers to conduct many *in vivo* trials to study the effects of grape products (grape juice, grape seed, grape skin, polyphenol-rich extracts) on different CVD risk factors. Most reviews have focused only on wine[7] or have dealt with some specific aspects of grape and wine flavonoids' mechanisms of action, such as their effects on endothelial dysfunction[8]. However, no paper has systematically discussed the *in vivo* trials performed during recent years in relation to grape products and CVD risk.

The aim of the present paper is to review the trials published during the last 13 years (thirty-four in animals and forty-one in human subjects) examining the effects of supplementation with grape products on CVD risk factors.

Grape products used in clinical trials

Clinical trials have evaluated the *in vivo* effects of grapes, wine, grape skin, grape seeds, grape pomace and grape polyphenol extracts. Table 1 summarises the main compositional characteristics of these products.

Grapes (*Vitis vinifera* L.) contain high concentrations of polyphenols, especially flavonoids. The amount and composition of biologically active compounds present in grapes and grape products vary greatly according to the

---

Table 1. Main compositional characteristics of grapes and polyphenol-rich derived products[9,14,28,30,34]

Product	Main polyphenols	Other components
Grapes	Anthocyanidins in red grapes, flavan-3-ols in white grapes	Sugar (16 % fresh matter) Dietary fibre (about 1 % fresh matter)
Wine	Flavan-3-ols, flavan-3,4-diols, anthocyanins, flavones, condensed tannins, resveratrol. Content is higher in red wine than in white wine	Ethanol (3·5–13·5 %)
Grape juice	Anthocyanins in red grape juice, flavan-3-ols in white grape juice, ellagic acid	Sugar (16 %)
Grape skin	Prodelphinidins	Dietary fibre (> 75 %)
Grape seeds	Procyanidins	Dietary fibre (> 75 %)
Grape pomace	Anthocyanins, anthocyanin-derived pigments	Dietary fibre (> 60 %)
Polyphenol extract	Anthocyanidin-3-glucosides	Main components are polyphenols; others are usually non-identified

species, variety, maturity, seasonal conditions, production area and yield of the fruit[9]. The main grape polyphenols are anthocyanins in red grapes and flavan-3-ols in the case of white grapes; red grapes contain more total polyphenols than white grapes. Grape seeds and skins are also an important dietary source of flavonoids, and seeds contain significant amounts of proanthocyanidins or condensed tannins. The composition of the seeds is much more diverse than that of the skins[9]. Some *in vivo* studies have dealt with the effects of the intake of the original grapes after freeze-drying[10,11]. Other studies have investigated the effects of the intake of grape seeds or grape peels separately[12–14].

The most common commercial product derived from grapes is wine, a moderately alcoholic drink made by fermentation of juice extracted from fresh, ripe grapes. The processing of grapes to yield wine transforms the polyphenols present in grapes, and as a result the main polyphenols in wine are flavan-3-ols, flavan-3,4-diols, anthocyanins and anthocyanidins, flavonols, flavones, condensed tannins and a characteristic biologically active compound, resveratrol – a stilbene whose concentration can range from 1·5 to 3 mg/l[9,15]. Consequently, given the polyphenol contents of the raw material, red wine exhibits a much higher phenolic content than white wine. Several *in vivo* studies have examined the effects of the intake of red[16–19] and white[20,21] wine.

There are some alcohol-free products derived from wine. The most common of these is grape juice, which has been used in several *in vivo* studies[22–24]. Grape juice differs little in composition from grapes except that it lacks dietary fibre and oil. Its characteristic polyphenols include ellagic acid, an acid hydrolytic product of ellagitannins[9]. Also, for some intervention studies dealcoholised red wine has been prepared[12,25] in order to consider the possible effect of ethanol on the bioavailability of polyphenols and to differentiate the positive effects on CVD due to polyphenols and those due to ethanol. To the authors' knowledge, no *in vivo* study has considered solely the possible positive effects of dealcoholised white wine.

Because grape juice has a high energy value due to its high sugar content, other products derived from grapes have been studied. The most common of these are products derived from grape pomace, a by-product of wine-making consisting of pressed skins, seeds and stems. Traditionally, grape pomace was used as a source of different products, such as ethanol, tartrates, citric acid, grape seed oil, grape

seed tannins, hydrocolloids and anthocyanins[9]; however, with growing interest in the beneficial effects of grapes over the last few years, grape pomace-derived products have come to be used in intervention studies[26,27] because of its high concentrations of phenolic compounds and dietary fibre, another beneficial component[28]. Specific preparations of grape products rich in both dietary fibre and polyphenols have also been used to check the possible combined effect of these compounds[29].

Finally, several commercial products have been developed during the last few years, mainly in the form of pills. A recent study[30] compared the antioxidant capacity of up to thirteen commercial products derived from grape skins, grape pomace and grape leaves; most of these products are supplied in solid form, although some of them came in the form of liquid concentrates and syrups. Anthocyanidin-3-glucosides presented the largest concentration, although the profiles of the ingredients derived from skin and those derived from leaves were different. However, in this and in other studies[31] it has been noted that the polyphenol content of these supplements differs significantly, which means that the biological activities derived from them will be different. Some of them have been employed in *in vivo* studies[32–33].

In short, different compounds may contribute to the observed effects depending on the tested grape product: polyphenols and ethanol in wine, polyphenols and sugar in grape juice, polyphenols and dietary fibre in grape seeds and grape skins, or just polyphenols in extracts[9,15,28,30,34].

Trials reviewed

Table 2 summarises the selected *in vivo* studies performed on animals. Thirty-four articles have been considered. They include studies on supplementation with freeze-dried grapes, grape pomace, grape peel, grape seed, polyphenols from grapes, polyphenols from grape seed, extracts from white grape seed, extracts from red grape seed, a grape product rich in both dietary fibre and polyphenols, grape juice, red and white wine, dealcoholised red wine, red wine powder and red wine extracts. The studies were performed on rats, apoE-deficient mice, rabbits (normal and Watanabe heritable hyperlipidaemic), hamsters, gerbils, ovariectomised guinea-pigs, chickens, monkeys and dogs. The number of animals in each study was between twenty and 180, with an average of fifty-three subjects. The duration of

Table 2. Published trials in animals on effects of grapes, wine and derived products on CVD risk factors in animals*

Reference	Species and number of animals	Duration	Product and intake
Cui et al. (2002)[10]	Rats		Control treatment or freeze-dried grapes at different concentrations: 50, 100 and 200 mg/kg weight per d
Zern et al. (2003)[11]	Twenty-three ovariectomised guinea-pigs	12 weeks	Control treatment or diet with 10 % freeze-dried grapes, 6 % dietary fibre and 0·5 % total polyphenols. Intake ad libitum
Ruf et al. (1995)[12]	144 rats	4 months	Series 1: tap water, ethanol 6 %, red wine diluted in water (6 % ethanol) or white wine diluted in water (6 % ethanol) Series 2: ethanol 6 %, red wine diluted in water (6 % ethanol), ethanol 6 % with 0·2 % glycerol or ethanol 6 % with 0·025 % grape seed extract Series 3: ethanol 6 %, red wine diluted in water (6 % ethanol) or red wine extract added to ethanol 6 % Series 4: water, red wine diluted in water (6 % ethanol) with the same content in glycerol as dealcoholised red wine or dealcoholised red wine
Martín-Carrón et al. (2000)[13]	Twenty-four rats	4 weeks	Diet with and without cholesterol, two groups in each: control treatment or product from grapes rich in polyphenols (1·63 %) and fibre (50 %) (10 % diet)
Martín-Carrón et al. (2000)[13]	Eighty rats	42 d	Diet with and without cholesterol, three groups in each: control with cellulose, white grape peel or white grape seed (all the groups with 54–59 % dietary fibre)
Falchi et al. (2006)[14]	Rats	30 d	Grape seed extract (0·18–0·33 % total polyphenols fresh matter), grape flesh extract (0.03–0·04 % total polyphenols fresh matter) or water
Osman et al. (1998)[22]	Five monkeys	1 week	Orange juice, grape juice or grapefruit juice (5 ml/kg per d of each) Cross-over design
Shanmuganayagam et al. (2007)[23]	Twenty rabbits	48 d	Diet with cholesterol in all groups. Water with sugar or grape juice with 1·97 g total polyphenols/l
Bobek et al. (1998)[26]	Eighty rats	10 weeks	Diet with cholesterol supplemented with: cellulose, grape pomace, apple pomace or tomato pomace
Diebolt et al. (2001)[40]	Twenty-four rats	1 week	Product with glucose 5 % or two different extracts of polyphenols from red wine (in all cases, 20 mg/kg weight per d)
Bernatova et al. (2002)[41]	Forty-eight rats	After taking L-NAME for 3 weeks, three groups: Six were killed Eighteen were divided into three groups and came back to their usual diet after 1, 2 and 3 weeks Eighteen were divided into three groups and were supplemented for 1, 2 and 3 weeks	Control treatment or red wine polyphenols
Soares de Moura et al. (2002)[42]	Rats	28 d in hypertensive rats providing L-NAME and 13 d in hypertensive rats providing deoxycorticosterone	Control treatment or grape peel with 5·5 % total polyphenols (100 mg/kg weight per d)
Al-Awwadi et al. (2004)[43]	Forty-five rats	6 weeks	Control treatment, diet rich in fructose, diet rich in fructose and red wine extract in water with 47·1 % total polyphenols, diet rich in fructose and ethanol 10 % or diet rich in fructose and red wine extract in ethanol with 47·1 % total polyphenols (all supplementations with 10 ml/kg weight per d)
Ranaivo et al. (2004)[44]	Rats	1 week	Supplementation by intragastric administration: control with glucose 5 %, RWPC (20 mg/kg weight per d), L-NAME or RWPC and L-NAME
Wollney et al. (1999)[50]	Rats	10 d	Red wine with 12 % ethanol, dealcoholised red wine, ethanol solution (12 %) or white wine
Vinson et al. (2001)[58]	Hamsters	10 weeks	Water, ethanol, red wine, dealcoholised red wine or grape juice

Auger *et al.* (2002)[59]	Thirty-two hamsters	8 weeks	Atherogenic diet supplemented with: 7·14 ml water/kg weight per d, 7·14 ml ethanol/kg weight per d, diet with 47·1 % red wine polyphenols in water or diet with 47·1 % red wine polyphenols in ethanol
Auger *et al.* (2004)[60]	Forty hamsters	12 weeks	Diet with cholesterol in all groups. Control treatment, extract from white grape seed rich in procyanidins, extract from white and red grape seeds rich in procyanidins or extract from grape seeds (in all supplemented groups, 7·14 ml/kg weight per d)
Auger *et al.* (2005)[61]	Hamsters	12 weeks	Atherogenic diet in all groups. Control treatment, ethanol, red wine (570 mg total polyphenols/l) or enriched white wine (1425 mg total polyphenols/l)
Cestaro *et al.* (1996)[62]	Forty rats	4 weeks	Red wine (12 % ethanol), dealcoholised red wine with sugar, ethanol 12 % or water with sugar
Yamakoshi *et al.*(1999)[63]	Thirty-eight rabbits	8 weeks	Control treatment, diet with cholesterol, diet with cholesterol and 1 % extracts from grapes, diet with cholesterol and 0·1 % extracts from grapes or diet with cholesterol and Probucol
Araya *et al.* (2001)[64]	Eighty rats	10 weeks	Water, ethanol 12·5 %, red wine with 12·5 % ethanol or dealcoholised red wine
Pal *et al.* (2004)[73]	Twenty rats	5 weeks	Control with cellulose or grape product rich in both dietary fibre and polyphenols with 4·7 % total polyphenols (50 mg/kg weight per d)
Hayek *et al.* (1997)[70]	Forty apoE-deficient mice	6 weeks	Ethanol 1·1 %, 0·5 ml red wine/d (1·1 % ethanol and 50 μg catechin), 50 μg catechin/d in ethanol 1·1 % or 50 μg quercetin/d in ethanol 1·1 %
Nakamura & Tonogai (2002)[72]	Rats	28 d or 36 d (two experiments)	Diet with and without cholesterol and with different doses of grape seed polyphenols
Frederiksen *et al.* (2007)[83]	Twenty-seven Watanabe heritable hyperlipidaemic rabbits	15 weeks	Semi-synthetic diet or semi-synthetic diet with 2·6 % extract from red grape skin and seed
Waddington *et al.* (2004)[84]	100 apoE-deficient mice and fifty control mice	15 weeks	Water in controls, water in apoE-deficient mice or red wine in apoE-deficient mice (0·6 mg polyphenols/d)
Benteon *et al.* (2001)[85]	Eighty-four apoE-deficient mice	21 weeks	Water, red wine (ethanol 6 %), ethanol 6 % or red wine powder
Stocker & O'Halloran (2004)[86]	Eighty-seven apoE-deficient mice	24 weeks	Water or dealcoholised red wine
Xia *et al.* (1998)[87]	Rats	2 months	Control, ethanol, polyphenols from grapes and ethanol or polyphenols from grapes
Rodrigo *et al.* (2002)[93]	Eighty rats	10 weeks	Red wine, dealcoholised red wine, water, ethanol or ethanol 12·5 %
Goñi *et al.* (2007)[95]	120 chickens	3 weeks	Vitamin E (200 mg/kg weight per d), red grape pomace (5 mg/kg weight per d) or control with cellulose (30 mg/kg weight per d)
Brenes *et al.* (2008)[96]	180 chickens	3 weeks	Control, vitamin E (200 mg/kg weight per d), red grape pomace concentrate (15 mg/kg weight per d), red grape pomace concentrate (30 mg/kg weight per d) or red grape pomace concentrate (60 mg/kg weight per d)
Roig *et al.* (1999)[101]	Fifteen rats	45 d or 6 months (two experiments)	Water, ethanol 13·5 % or red wine with 13·5 % ethanol. Intake *ad libitum*
Wang *et al.* (2005)[102]	Fifty gerbils	2 months	Control treatment or freeze-dried grapes (5 g/kg diet or 50 g/kg diet)

L-NAME, *N*-nitro-L-arginine-methyl ester; RWPC, red wine polyphenols concentrate.
* When not indicated expressly, grapes are red grapes and wine is red wine.

the studies was between 1 week and 6 months, with an average of 2 months.

Table 3 summarises the selected *in vivo* studies performed on human subjects. Forty-one articles have been considered. They include studies on supplementation with freeze-dried grapes, grape peel, grape seed, polyphenols from grapes, polyphenols from grape seed, grape juice, concentrated grape juice, red and white wine, dealcoholised red wine and red wine polyphenol extracts. The studies include healthy subjects, subjects with coronary artery disease, type 2 diabetic patients, pre- and postmenopausal women, hypercholesterolaemic subjects, hypertensive subjects, men with stable IHD and haemodialysed subjects. The number of participants in each study was between eight and sixty-nine, with an average of twenty-four subjects. The duration of the studies was between 1 and 16 weeks, with an average of 25 d, plus eight studies which were based on an acute intake.

Effects on blood pressure: endothelial function

The US National High Blood Pressure Education program estimates that a reduction of 5 mmHg in systolic blood pressure (SBP) translates into a 14 % reduction in deaths by stroke, a 9 % reduction in deaths by heart disease and a 7 % reduction in overall mortality[35]. This has generated an interest in the search for new bioactive dietary compounds that can reduce blood pressure, among them polyphenols.

However, when considering the effects of grape products on blood pressure, the ethanol content of red wine, one of the main grape products, should not be disregarded. It is well known that there is a linear relationship between alcohol intake and blood pressure[36]. Although several epidemiological studies have suggested that beer and spirits consumption may be associated with higher blood pressure (particularly SBP) than wine consumption[37,38], an *in vivo* trial[39] showed that daily consumption of 40 g alcohol as either red wine or beer for 4 weeks resulted in similar increases in SBP and heart rate. Therefore, studies on the effects of grape products on blood pressure should be focused more on grape products lacking ethanol.

All the reviewed studies on animals dealing with the effects of grape polyphenols on blood pressure showed a hypotensive effect of them. Grape skin extract, red wine polyphenols and red wine extract significantly reduced blood pressure (in several cases, both SBP and diastolic blood pressure) in normotensive and hypertensive rats, where hypertension was induced by *N*-nitro-L-arginine-methyl ester (L-NAME) or by deoxycorticosterone acetate[40–44].

In the case of human subjects, a significant reduction in SBP and diastolic blood pressure was observed in hypertensive or coronary artery disease subjects after the intake of grape juice[45]. Also, the intake of a grape product rich in both dietary fibre and polyphenols by normotensive subjects for 16 weeks led to a non-significant decrease in SBP and diastolic blood pressure[30]. This is an interesting result, since assays on the effects of supplementation on blood pressure are usually performed on hypertensive subjects, and these might be expected to present a more marked effect if administered this product, which is rich in both polyphenols and dietary fibre. However, another study did not find any effect of dealcoholised red wine on blood pressure[39]. Only

one of the reviewed trials found that grape polyphenols had adverse effects on blood pressure; there, increased blood pressure was observed in hypertensive subjects when polyphenols were combined with vitamin C[46].

As to mechanisms that would explain the generally observed hypotensive effect of polyphenols, it has been suggested that they promote the release by the vascular endothelium of NO, a compound with vasorelaxing and anti-aggregating effects and that in the long term induces the expression of protective genes for the cardiovascular system[40,47]. It has also been proposed that wine may regulate the endothelial NO synthase gene in endothelial cells, through transcriptional and post-transcriptional factors[48].

These hypotheses have only been partially proven. In an *ex-vivo* experiment with aortic rings from rats incubated with red grape polyphenols, there was a two-fold increase in the $NO-Fe(diethyldithiocarbamate)_2$ electron paramagnetic resonance, which indicates the generation of NO; this NO production was completely prevented by the NO synthase inhibitor L-NAME[49]. Similarly, after supplementing rats with red wine and observing an anti-thrombotic effect[50], the effects produced by red wine were prevented by L-NAME, indicating the involvement of NO in this process. Moreover, the effects of L-NAME could be reverted by L-arginine, the precursor of NO synthesis in vascular endothelium but not by its stereoisomer, D-arginine. In human subjects, although an acute intake of polyphenols did not significantly increase NO production[51], the supplementation to healthy volunteers with grape juice for 14 d led to a significant increase in platelet-derived NO production (from 3·5 (SEM 1·2) to 6·0 (SEM 1·5) pmol/10^8 platelets[52]).

Another parameter that has been studied to determine the effect of grape polyphenols on endothelial function has been the determination of effects on vasodilatation and, particularly, on flow-mediated dilatation of the brachial artery, which is considered to be an early marker of alterations in endothelial function. In studies in animals, red wine polyphenols induced endothelium-dependent relaxation in rat aorta[49], red wine and dealcoholised red wine induced vasodilatation in isolated vessels from rats[53] and grape skin extract had a vasodilator effect on the mesenteric cardiovascular bed of rats[42]. In human subjects, an improvement in endothelium-dependent vasodilatation has been reported after the acute intake of white wine or red wine with a meal by subjects with IHD[54]; also it has been observed that an acute intake of red grape polyphenol extract, red wine or dealcoholised red wine cause an increase in flow-mediated dilatation, peaking at 60 min[54,55]. Nevertheless, in another study[53] red wine, but not polyphenols from red wine, produced an enhancement of endothelial response, despite a similar catechin concentration in both products. Intake of grape juice or grape seed extract for 2–3 weeks also caused a significant increase in flow-mediated dilatation, compared with the control group[56,57]. Interestingly, in one of these studies[57], the addition of quercetin to the grape seed extract nullified this effect, indicating maybe that the provided amount of antioxidants was excessive, thus becoming pro-oxidants.

Also, grape polyphenols may have a reducing effect in cardiac fibrosis, a process occurring in cases of hypertension

Table 3. Published trials on effects of grapes, wine and derived products on CVD risk factors in human subjects*

Reference	Participants	Duration	Product and intake
Badia *et al.* (2004)[16]	Eight healthy men	2 weeks	Red wine with 12·5 % ethanol (320 ml/d) or gin (100 ml/d) (in both cases, 30 g ethanol/d)
Sacanella *et al.* (2007)[17]	Thirty-five healthy women	4 weeks	Red wine with 13·5 % ethanol and 1·9 g total polyphenols/l or white wine with 13 % ethanol and 0·31 g total polyphenols/l
Agewall *et al.* (2000)[18]	Twelve healthy subjects	Acute intake	Red wine with 1·9 g total polyphenols/l and 12·5 % ethanol or dealcoholised red wine with 1·1 g total polyphenols/l (250 ml in all groups) Cross-over design
Naissides *et al.* (2004)[19]	Seventeen postmenopausal women	Acute intake	Dealcoholised red wine with 2·2 g total polyphenols/l, white wine with 2·2 g total polyphenols/l or water Cross-over design
Williams *et al.* (2004)[20]	Fourteen men with stable IHD	Acute intake	Red wine with 13·5 % ethanol and 1·2 g total polyphenols/l, white wine with 13 % ethanol and 0·2 g total polyphenols/l or non-alcoholic drink without polyphenols Cross-over design
Pignatelli *et al.* (2006)[21]	Twenty healthy subjects	3 weeks	Red wine with 12·5 % ethanol and 1·2 g total polyphenols/l or white wine with 12·5 % ethanol and 0·18 g total polyphenols/l
Castilla *et al.* (2008)[24]	Thirty-two haemodialysed patients	2 weeks	Red grape concentrate (100 ml/d = 600 mg total polyphenols/d), 800 IU (20 μg) vitamin E/d or both
De Rijke *et al.* (1996)[25]	Twenty-four healthy subjects	2 weeks, then 4 weeks with different treatments	After 2 weeks with white wine: 4 weeks with white wine (3·5 % ethanol) or partially dealcoholised red wine (3·5 % ethanol) (550 ml/d in all groups)
Pérez-Jiménez *et al.* (2008)[29]	Forty-three non-smokers, including normocholesterolaemic and hypercholesterolaemic subjects	16 weeks	Control treatment or grape product rich in both dietary fibre and polyphenols (7·5 g/d), with 4·93 % total polyphenols and 73·5 % dietary fibre
Carbonneau *et al.* (1997)[32]	Twenty healthy men	14 d	Six daily pills containing an extract of red wine polyphenols
Vinson *et al.* (2001)[33]	Nine hypercholesterolaemic and eight normocholesterolaemic subjects	3 weeks	MegaNatural® Gold Grape Seed Extract (600 mg extract = 552 mg total polyphenols)
Zilkens *et al.* (2005)[39]	Twenty-four healthy men who drank 30−60 g alcohol/d	4 weeks	Red wine with 13 % ethanol and 2 g total polyphenols/l (375 ml/d), the same wine dealcoholised (375 ml/d), deprivation of alcohol and grapes or beer with 4·6 % ethanol (1175 ml/d)
Park *et al.* (2004)[45]	Hypertensive men	8 weeks	Grape juice with 2·1 g total polyphenols/l or placebo (11 ml/kg weight per d in all groups)
Ward *et al.* (2005)[46]	Hypertensive subjects	6 weeks	Vitamin C, polyphenols from red grape seeds (1 g/d), both or control
Chou *et al.* (2001)[47]	Fifteen healthy subjects	1 week	Extract from grape peel with 5 % total polyphenols (600 mg extract/d)
Matsuo *et al.* (2001)[51]	Six men	Acute intake	Red wine, ethanol, polyphenols from red wine Cross-over design
Freedman *et al.* (2001)[52]	Twenty healthy volunteers	2 weeks	Purple grape juice (7 ml/kg weight per d)
Boban *et al.* (2006)[53]	Nine men	Acute intake	Red wine, dealcoholised red wine, polyphenols from red wine, ethanol−water solution, water Cross-over design
Whelan *et al.* (2004)[108]	Fourteen men with stable IHD	Acute intake	Red wine with 13·5 % ethanol and 1·2 g total polyphenols/l or white wine with 13 % ethanol and 0·2 g total polyphenols/l Cross-over design
Hashimoto *et al.* (2001)[54]	Nine men	Acute intake	Water, Japanese vodka (shochu, 0·8 g ethanol/kg weight), red wine with 0·8 g ethanol/kg weight or dealcoholised red wine Cross-over design
Lekakis *et al.* (2005)[55]	Thirty men with CHD	Acute intake	Red grape polyphenols (600 mg) or water
Stein *et al.* (1999)[56]	Fifteen subjects with coronary artery disease	2 weeks	Grape juice (4 ml/kg weight per d)
Clifton (2004)[57]	Forty-three subjects with above-average vascular risk	4 weeks	Grape seed extract (2 g/d), grape seed extract (2 g/d) plus quercetin (0·5 g/d) or control
Castilla *et al.* (2006)[65]	Thirty-eight haemodialysed and fifteen healthy subjects	2 weeks	Concentrated grape juice (100 ml/d = 600 mg total polyphenols/d)
Cordain *et al.* (2000)[66]	Twenty sedentary and overweight premenopausal women	10 weeks	Red wine with 13 % ethanol (190 ml/d) or absence of alcohol Cross-over design

Table 3. *Continued*

Reference	Participants	Duration	Product and intake
Cacetta *et al.* (2001)[67]	Eighteen male smokers	2 weeks	Red wine with 13·3 % ethanol and 1·2 g total polyphenols/l (375 ml/d), white wine with 13·7 % ethanol and 0·34 g total polyphenols/l (375 ml/d) or dealcoholised red wine with less than 2 % ethanol and 0·9 g extractable polyphenols/l (500 ml/d)
Zern *et al.* (2005)[68]	Twenty-four premenopausal and twenty postmenopausal women	4 weeks	Placebo or freeze-dried red grapes with 3·4 % dietary fibre and 5·8 % total polyphenols Cross-over design
Naissides *et al.* (2006)[69]	Forty-five postmenopausal women with moderate hypercholesterolaemia	6 weeks	Water, red wine with 13 % ethanol and 2·5 g total polyphenols/l or dealcoholised red wine with 2·5 g total polyphenols/l (400 ml/d in all groups)
Fuhrman *et al.* (1995)[75]	Seventeen healthy men	2 weeks	White wine or red wine (400 ml/d in all groups)
Hansen *et al.* (2005)[76]	Sixty-nine healthy subjects	4 weeks	Red wine with 12·75 % ethanol (300 ml/d for men and 200 ml/d for women), equivalent dose of grape polyphenols, half the equivalent dose of grape polyphenols or placebo
Tsang *et al.* (2005)[77]	Twenty-three healthy subjects	2 weeks	Red wine 12 % ethanol (375 ml/d = 165 mg polyphenols) or control
Avellone *et al.* (2006)[78]	Forty-eight healthy subjects	4 weeks	Red wine (250 ml/d)
O'Byrne *et al.* (2002)[80]	Thirty-six healthy subjects	2 weeks	Grape juice concentrate (10 ml/kg weight per d) or 400 IU (268 mg) RR-α-tocopherol
Keevil *et al.* (2000)[81]	Ten healthy subjects	1 week	Grape juice, grapefruit juice or orange juice (5–7·5 ml/kg weight per d) Cross-over design
Pace-Asciak *et al.* (1996)[82]	Twenty-four healthy men	4 or 8 weeks with different treatments	Series 1: 2 wash-out weeks +4 weeks with red wine +2 wash-out weeks +4 weeks with white wine

Series 2: 2 wash-out weeks +8 weeks with enriched grape juice +2 wash-out weeks +4 weeks with white wine +2 wash-out weeks +4 weeks with red wine
All supplementations with 375 ml/d |
Nidgikar *et al.* (1998)[88]	Thirty healthy men	2 weeks	Red wine with 1·6 g total polyphenols/l, white wine with 0·2 g total polyphenols/l, white wine and 1 g polyphenols, 1 g red wine polyphenols or control drink with ethanol 10 % (375 ml/d in all groups) Cross-over design
Nidgikar *et al.* (1998)[88]	Twenty healthy men	2 weeks	Blackcurrant drink (330 ml/d), 1 g red wine polyphenols, 2 g red wine polyphenols or 1000 IU (671 mg) RR-α-tocopherol Cross-over design
Estruch *et al.* (2004)[89]	Forty healthy subjects	4 weeks	Red wine (320 ml/d) or gin (100 ml/d)
Van der Gaag *et al.* (2000)[92]	Eleven healthy subjects	3 weeks	Red wine, beer, spirits or mineral water (four glasses/d in all groups) Cross-over design
Simonetti *et al.* (2002)[98]	Ten healthy subjects	1 month	Pills with procyanidins from grape seed (280 mg/d)
Ceriello *et al.* (2001)[100]	Twenty type 2 diabetic patients	Acute intake	Standard meal test, fasting ingestion of red wine (300 ml) or meal plus red wine (300 ml)
Guarda *et al.* (2005)[103]	Twenty patients with acute coronary syndrome	2 months	Red wine (250 ml/d)
Gin *et al.* (1999)[107]	Thirty men with non-insulin-dependent diabetes mellitus	Acute intake	Control treatment, 200 ml red wine, 150 mg tannic acid (equivalent to the content in 200 ml red wine) or 16 g ethanol (equivalent to the content in 200 ml red wine)

* When not indicated expressly, grapes are red grapes.

which is produced by an excessive accumulation of collagen and is associated with an increase in alterations of cardiac and vascular functions[41].

Effects on lipid profile

Several studies have focused on the possible positive effects of grape products on different lipid profile parameters, particularly total cholesterol, and likewise on LDL- and HDL-cholesterol, TAG and apolipoproteins. The results of these trials are summarised in Table 4.

Total cholesterol, LDL-cholesterol and HDL-cholesterol

Nine of the reviewed trials in animals reported a positive effect of grapes and derived products on plasma total cholesterol. This was observed after supplementation with grapes, grape pomace, grape juice, red grape skin, white grape seed, red and white grape extracts rich in procyanidins, red wine, red wine polyphenols, dealcoholised red wine or white wine enriched with red wine polyphenols, to rats fed with a cholesterol-rich diet, to hamsters (fed with a normal or with an atherogenic diet), to ovariectomised guinea-pigs or to rabbits[11,13,23,27,58–61].

Four similar studies in animals reported no effect on plasma total cholesterol[26,62–64]. So, it cannot be said overall that grapes and derived products present positive effects on plasma cholesterol in animal studies. However, one of these studies[26] did find a reduction in cardiac cholesterol and in the half-times of serum [14C]cholesterol decay curves, while another reported a decrease in aorta total cholesterol[63].

As regards studies in human subjects, although a significant reduction in total cholesterol has been observed, for example of 12 % after the intake of a grape seed extract[58] or of 6–11 % after the intake of concentrated grape juice[24,65], many other studies found no change in this parameter after the intake of grapes or derived products[21,25,47,52,66,67].

In the case of LDL-cholesterol, significant reductions have been observed in studies in both animals[13,27] and in human subjects[24,29,33,65,68,69], although other studies have reported no effect either in animals[11,70] or in human subjects[21,24,47,66,67].

The differences in the results of these assays may have to do with the design of the study, the polyphenol content of the tested product or its storage conditions. For example, it has been noticed that the higher the initial concentration of plasma cholesterol, the greater the reduction[29,33,69]. This would explain the lack of effects in some of the cited studies in human subjects, which targeted normocholesterolaemic subjects. The importance of a proper design can be seen from the fact that in the studies where some change has been observed in lipid profile, this change has affected several parameters[26,64].

The main mechanism responsible for this hypolipidaemic effect would be reduction in intestinal cholesterol absorption, leading to an enhanced excretion of faecal neutral steroids and bile acids. This was first observed by Tebib et al.[71] in rats supplemented with low- and high-molecular-weight tannins (a class of polyphenols) from grapes and was later observed in other assays, for example, in rats supplemented with a grape seed extract[72]. Similarly, polyphenols would reduce the intestinal absorption of dietary fat, since it has been observed that both normal and dealcoholised red wine reduced the postprandial concentrations of chylomicrons[73]. Also, it has been observed that polyphenols, in this case from tea, with high molecular weight (such as tannins present in grapes) form complexes with bile, which causes disruption of micelles, leading to the precipitation of cholesterol in the intestinal lumen, which is a behaviour similar to that of water-soluble dietary fibre[74].

It is also interesting to note that other observed facts besides changes in plasma total cholesterol may also have a positive effect on lipid metabolism. For example, after supplementing of ovariectomised guinea-pigs with freeze-dried grapes, a positive change was found in the concentration and composition of LDL particles that were not readily taken up by the aorta, resulting in less accumulation of cholesterol in this tissue[11]. The authors of this study also suggested that grapes may reduce the secretion of LDL particles by the kidney.

As regards HDL-cholesterol, although some studies in animals have reported positive effects after supplementation with alcohol-free products[13,27,64], in studies in human subjects increased HDL-cholesterol has only been observed once after the intake of red grape juice concentrate by haemodialysed patients[24], but did not take place in the trials with alcohol-free grape products[69,75–78]. Therefore, this effect is presumably mainly related to the already-known ability of ethanol to increase HDL-cholesterol[75] instead of polyphenol content.

Table 4. Summary of observed effects of grapes, wine and derived products on lipid profile in the reviewed studies

	Parameter	Trials with positive effects*	Trials with neutral effects*	Negative effects*
Studies on animals	Total cholesterol	13, 73, 58–61	26, 62–64	
	LDL-cholesterol	13, 27	11, 70	
	HDL-cholesterol	13, 27, 64	61, 62, 70	
	TAG	11, 13, 72, 86		64
	Apolipoproteins	59, 61	60	
Studies on human subjects	Total cholesterol	24, 29, 58, 65	21, 25, 51, 66, 67	57
	LDL-cholesterol	24, 29, 65, 68, 69	21, 25, 32, 47, 65, 66, 69	
	HDL-cholesterol	69, 75–78	21, 25, 32, 47, 66–68	
	TAG	29, 68	21, 25, 47, 65–67	19, 56, 80
	Apolipoproteins	24, 65, 68	19	

* Numbers correspond to references in the Reference section.

Triacylglycerols

It is known that ethanol can increase plasma TAG[79]. One would therefore expect that if grapes and derived products had a positive effect on plasma TAG, this would only be so in the case of alcohol-free products. This tendency was observed in studies in animals, when supplementation with freeze-dried grapes, white grape peel, white grape seed, grape seed polyphenols or dealcoholised red wine to rats, ovariectomised guinea-pigs or apoE-deficient mice reduced plasma TAG[11,13,72], while supplementation with wine had neutral or negative[64] effects on this parameter.

Nevertheless, this aspect was less clear in the case of human subjects. Although supplementation to menopausal women with freeze-dried grapes reduced plasma TAG by up to 15 %[68] and the intake of a grape product rich in both dietary fibre and polyphenols had a similar effect on hypercholesterolaemic subjects[29], the intake of other non-alcoholic grape-derived products had neutral[21,24,47,65] or negative[56,80] effects. Interestingly, the two tested products that had a positive effect on this parameter contained dietary fibre, and that may have contributed significantly to this effect.

Apolipoproteins

The effects of grapes and derived products on apolipoproteins have not been systematically studied. However, the results of a few trials have shown that the hypolipidaemic effect of grape polyphenols may be related to their effects on the concentrations of certain apolipoproteins. For instance, red wine or red wine polyphenol supplementation to rats increased apoA1 and reduced apoB[59,61]. Similarly, the same effects were produced by the intake of freeze-dried grapes or concentrated grape juice by menopausal women or by healthy and haemodialysed subjects respectively[24,65,68].

Effects on platelet aggregation

Reducing platelet activity is a preventive strategy to reduce the development of atherosclerosis[23]. Several trials have studied the possible effects of grapes, wine and derived products on platelet aggregation.

In studies in animals, it has been observed that the intake of grape juice reduces platelet aggregation induced by collagen or by ADP as agonists, but not aggregation induced by phorbol-12-myristate-13-acetate[22,23].

In a trial in rats on the rebound effect on thrombin-induced platelet aggregation after alcohol withdrawal, it was observed that 18 h after deprivation of alcohol, 6 % ethanol increased the platelet response by 124 %, white wine by 46 %, and red wine reduced it by 59 %. This is an interesting aspect, since the increase in platelet aggregation after alcohol deprivation is sometimes associated with sudden death and stroke in humans[12].

The intake of grape juice by human subjects reduced platelet aggregation induced by collagen, ADP and phorbol-12-myristate-13-acetate; it also caused an increase in platelet-derived NO release and a decrease in platelet superoxide anion production[52,81]. The intake of a grape juice enriched in resveratrol also reduced thrombin-induced platelet aggregation, but this effect was absent after supplementation with a commercial grape juice[82].

The intake of red wine and white wine by human subjects reduced thrombin-induced platelet aggregation and thromboxane B2 concentration, but only white wine reduced ADP-induced aggregation[82].

The suppression of platelet-mediated thrombosis, then, is another mechanism that could potentially explain the beneficial effects of grape polyphenols in CVD. Specific studies should be performed with grape skins, grape seeds, grape pomace and polyphenols extracted from grapes.

Anti-atherosclerotic effect

Atherosclerosis is a key pathology in CVD, which, once started, cannot be prevented but may be retarded[83]. Therefore, a great deal of research has focused on the positive effect that grapes and derived products may have in preventing the progression of this pathology.

Effects on atheromatous plaque

Several studies have been performed to evaluate the effect of supplementation with grape polyphenols in the development of atherosclerotic lesions. Due to the nature of the measurements performed, they can only be developed in animals.

A significant reduction in the size of atherosclerotic lesions or in the accumulation of foam cells in aorta has been observed after supplementation with red wine, dealcoholised red wine, red wine polyphenols, white wine enriched in polyphenols, and white and red grape extracts to apoE-deficient mice and hamsters with an atherogenic diet and to rabbits and Watanabe heritable hyperlipidaemic rabbits[58,61,63,83,84]. Also, red wine or dealcoholised red wine supplementation to rats induced a marked prolongation of template bleeding time, a decrease in platelet adhesion to fibrillar collagen and a reduction in thrombus weight, while neither ethanol nor white wine affected these systems[50].

These studies found that there was an effect mainly in the early stages of atherosclerosis, during fatty streak formation. In contrast, another study that examined the mature phase of atherosclerosis reported no positive effects in terms of the speed of progression of the lesions, inhibition in the aortic root and brachicephalic trunk or changes in collagen content of atheromatous plaques after red wine powder supplementation to apoE-deficient mice[85]. Similarly, Stocker & O'Halloran[86] found a reduction in the aortic lesions in the aortic arch, but not in the aortic root after supplementing apoE-deficient mice with dealcoholised red wine. Since lesions in the aortic root are more developed than in the aortic arch, this may confirm that grape polyphenols are only able to inhibit atherosclerosis during its early stages, although this would be an important beneficial effect in any case.

Anti-atherosclerotic and hypolipidaemic effects were considered separately in a study on hamsters supplemented with red wine or dealcoholised red wine[58]; it was observed that the polyphenols present in these products exhibited an anti-atherosclerotic effect over and above their effects on the

lipid profile. Also, these effects on the development of atheromatous plaque were presumably due not only to their antioxidant effects, but also to inhibition of cell proliferation in smooth muscle, regulation of adhesion molecules and reduction of the flux of atherogenic molecules from the endothelium to the arterial wall[52,53].

To the authors' knowledge, studies in animals have not specifically addressed the possible atherosclerotic effect of grape skins and grape seeds.

Although many of these assays cannot be performed on human subjects, some studies have examined the effect of wine intake on monocyte–endothelial cell adhesion, an early event in atheromatous plaque formation. Results show that white and red wine intakes reduced monocyte adhesion to TNF-α-stimulated endothelial cells by 51 and 89–96 % respectively[16,17]. Since the effect achieved by gin was 39 %, the polyphenols present in wine presumably contributed significantly to this effect, which may be the result of down-regulation of some monocyte adhesion molecules, especially very light-appearing antigen 4, on the monocyte surface.

Effects on LDL oxidation

The oxidative hypothesis of atherosclerosis suggests that LDL oxidation could be a first step in the development of atheromatous plaque. For instance, several studies have considered the effects of grape polyphenols on different parameters related to LDL oxidation.

A decrease in the concentration of oxidised LDL or an increase in the lag-phase in the oxidation of LDL has been observed after red wine or grape supplementation to apoE-deficient mice or to cholesterol-fed rabbits[63,70,85]. Interestingly, supplementation with wine polyphenols in ethanol to rats reduced LDL oxidation, but polyphenols in water had no effect and ethanol alone increased it, indicating a possible contribution of ethanol to the metabolism of polyphenols[87].

In studies in human subjects, the intake of red wine, red wine polyphenols, white wine enriched with red wine polyphenols, grape juice or concentrated grape juice by healthy subjects or haemodialysed patients had positive effects on LDL oxidation[21,65,77,78,80,88]. The effect of supplementation to human subjects with grape pomace on this parameter has not been considered.

However, other similar studies reported no such effects[25,33]. It is interesting that in one of these studies[47], the authors explained that the storage conditions of the tested product had not been the same as in a previous one where they had observed a decrease in LDL oxidation. This shows the importance of storage conditions for polyphenol-rich products, since it is well known that the antioxidant capacity of polyphenol-rich products decreases during storage at room temperature.

Castilla et al. [65] looked for a possible correlation between oxidised LDL and LDL concentration after the intake of grape juice by human subjects for 4 weeks; they did not find any, which suggests that besides a reduction in total LDL (the hypolipidaemic effect of polyphenols noted earlier), there may be other mechanisms – mainly antioxidants – that would explain the reduction in oxidised LDL.

Effects on inflammation markers

Inflammation and CVD are closely linked, since oxidised LDL are taken up by monocytes, starting the process that produces the foam cells characteristic of the onset of atherosclerosis.

Supplementation to apoE-deficient mice did not modify the levels of monocyte chemo-attractant protein 1 (MCP-1)[84]. However, studies in human subjects have mainly reported positive results regarding these parameters. The intake of red wine, but not of alcohol, reduced the expression of lymphocyte function-associated antigen 1, Mac-1, very light-appearing antigen 4 and MCP-1 in monocytes, and also the expression of vascular cellular adhesion molecule 1 and intercellular adhesion molecule 1 and the concentration of fibrinogen in lymphocytes[17,78,89]. Interestingly, one of these studies[17] reported these results in women who were supplemented with 20 g ethanol per d; although women's threshold of moderate ethanol consumption is lower than that of men, this dose was associated with beneficial effects in inflammation markers similar to those observed in men consuming doses of 30 g/d. Also, the intake of concentrated red grape juice by patients affected by end-stage renal disease treated with haemodialysis (with an increased oxidative stress) led to a reduction in plasma concentrations of MCP-1 and of NADH oxidase activity, a main source of superoxide radicals[24].

A significant increase was reported in IL-6 at 6 h after the intake of a meal accompanied by red or white wine, greater than the increase observed when the meal was accompanied by a control beverage[20]. The authors suggested that this increase was due more to a response to the hepatic damage induced by alcohol than to an activation of the inflammation response, since there was no change in the levels of cell adhesion molecules. Moreover, this increase in plasma IL-6 did not prevent a substantial improvement in endothelial function with wine intake.

However, other biomarkers have been proposed as being better suited to study the relationship between inflammation and CVD than cell adhesion molecules, specific cytokines or fibrinogen, which may be associated with any kind of inflammatory situation. The most important of these biomarkers are C-reactive protein and homocysteine[90,91]. A significant decrease in C-reactive protein has been observed after the intake of red wine[78,89], but not of freeze-dried grapes[68]. These studies did not express the content of polyphenols of the tested samples in the same way, which makes difficult a direct comparison between the results and to arrive at a possible explanation of the different effects observed. This disparity in the expression of polyphenol content is common in the reviewed trials and is a severe drawback when trying to compare them.

The results on homocysteine have also been contra-dictory; a non-significant decrease has been observed after the intake of red wine[77], while other studies have reported a significant undesired increase[92]. However, in this trial the subjects drank four glasses of wine daily, which is more than what is usually considered moderate.

In any event there is a need for more studies on the effects of grapes and derived products (particularly grape skins and grape seeds, products on which no study has been developed

in relation to inflammation) on inflammation markers, particularly C-reactive protein and homocysteine.

Effects on oxidative stress

There is a close link between a prolonged situation of oxidative stress and CVD, and dietary antioxidants may play a role in the prevention of the overproduction of free radicals. There are several ways to evaluate *in vivo* antioxidant status, including measurements of plasma antioxidant capacity, oxidation biomarkers, antioxidant compounds and evaluation of the activity of hepatic enzymes. Table 5 summarises the results of the trials reviewed in these parameters. It is interesting to note that the effect of all the grape-derived products considered in the present review on oxidative stress has been tested using more than one of the different common ways to evaluate it.

Plasma antioxidant capacity

The antioxidant capacity of plasma has been measured in different grapes and derived product supplementation studies in animals. An increase in the ferric-reducing antioxidant power value was observed in several studies after the intake of red wine or dealcoholised red wine by rats; the increase was greater with red wine[64,93]. Similarly, the intake of grape polyphenols by hamsters increased plasma antioxidant capacity measured by 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid); this increase was greater when polyphenols were dissolved in ethanol than when they were dissolved in water[59]. These results suggest that the bioavailability of polyphenols is enhanced in ethanol media, and hence in wine.

It has also been reported that there was an increase in the antioxidant capacity of rat and chicken faeces after supplementation with a grape product rich in both dietary fibre and polyphenols, and an increase in the excretion of polyphenols, particularly proanthocyanidins[94–96].

However, other authors have obtained results that contradict these. For example, one study found that the intake of red wine by apoE-deficient mice did not modify plasma antioxidant capacity[85]. The same happened after supplementation to hamsters with different extracts from grapes[52] or to rats with grape pomace. In this last case, there was actually a significant loss of antioxidant capacity in the liver[26]. These differences in results of similar assays may be related to the various limitations that have been reported in the determination of plasma antioxidant

capacity[97], as well as to differences in the designs of the studies (duration of treatment, doses employed).

In the case of human subjects, although some studies reported no effect on plasma antioxidant capacity after supplementation with grape-derived products[98], most of the trials did find some effect; for instance, increased plasma antioxidant capacity was observed after the intake of polyphenol extracts from red wine[32], from grapes[33], grape juice[65], concentrated grape juice[80], grape pomace[99] and red wine[77].

Also, studies on the possible effect of grape polyphenols in the postprandial phase have reported that after a meal accompanied by red wine, plasma antioxidant capacity by the total radical-trapping antioxidant parameter assay was sustained in type 2 diabetic subjects, whereas after the same meal without wine there was a significant decrease in antioxidant capacity[100].

A correlation was found between plasma antioxidant capacity and plasma cholesterol and TAG[85,100]. This is quite surprising, since subjects with higher plasma lipids and hence a higher risk of CVD would be expected to have lower values of plasma antioxidant capacity. This aspect requires further study, since it could be related to the transport of polyphenols within the bloodstream, a process that is not yet fully understood.

Oxidation biomarkers

Oxidation biomarkers are molecules that are directly affected by the oxidation process and hence furnish a more specific measurement of oxidative status.

Malondialdehyde (MDA) is a biomarker of lipid oxidation. The MDA concentration decreased in plasma, liver and kidney of rats supplemented with red wine for 6 months; in the group supplemented only with ethanol, MDA decreased in plasma in response to a higher consumption of vitamin E[101]. Similarly, freeze-dried grape supplementation to rats reduced MDA concentration in the heart[10], polyphenol-enriched red wine and white wine supplementation to hamsters reduced plasma MDA[61], and red wine and dealcoholised red wine supplementation to rats reduced the concentration of MDA in the renal cortex and renal papilla[93]. Grape extract supplementation to rabbits did not modify plasma MDA levels but did modify levels in the aorta[63]. Also, a reduction of MDA has been reported in breast and thigh meat from chicken supplemented with concentrated grape pomace[95,96].

Thiobarbituric acid-reactive substances (TBARS) are another marker of lipid oxidation, although less specific.

Table 5. Summary of observed effects of grapes, wine and derived products on oxidative stress

	Measurements	Positive effects†	Neutral effects†
Studies on animals	Plasma antioxidant capacity	59, 64, 93, 94	26*, 60, 85
	Biomarkers of oxidation	46, 61–63, 72, 88, 105	70
	Antioxidant compounds	62	86
	Antioxidant enzymes	26, 93, 101	26*
Studies on human subjects	Plasma antioxidant capacity	22, 32, 52, 65, 77, 80, 99	98
	Biomarkers of oxidation	21, 67, 68, 80, 98	25, 46, 80, 84
	Antioxidant compounds	21, 32, 65, 67, 77, 88, 98	25, 67

* Negative effects.
† Numbers correspond to refernces in the Reference section.

A decrease in the velocity of generation of TBARS was observed after supplementing rats with wine or with ethanol[62]. Similarly, grape seed polyphenol supplementation to rats reduced this marker by 33 %[72]. However, red wine supplementation did not modify this value in apoE-deficient mice[70].

In human studies, TBARS were reduced after the intake of white wine, white wine enriched with polyphenols and red wine polyphenols[88], although De Rijke et al.[25] reported no effect on this value after the intake of white or red wine. These differences may be due to the dose of polyphenols administered in each case; in the case of De Rijke et al.[25], the subjects drank more wine than in Nidgikar et al.[88] (550 v. 375 ml/d), but different polyphenol contents may have affected the results. It is not possible to determine this, since Nidgikar et al.[88] reported the total polyphenol content while De Rijke et al.[25] determined the content of only some particular polyphenols (catechins, quercetin, etc) in the wines tested, by HPLC. Many of the reviewed trials express the polyphenol contents in the tested substances differently, which makes it difficult to compare them directly.

Another suggested marker of lipid oxidation is F2-isoprostanes, although the results reported by different authors for this marker have been contradictory. It has been reported that there was a significant decrease in plasma and urine isoprostanes after the intake of red wine by male smokers[67], of freeze-dried grapes by pre- and postmenopausal women[68] and of white and red wine by healthy subjects[21]. Nevertheless, Waddington et al.[84] found no change in plasma isoprostanes or in hydroxyeicosatrenoic acids after red wine supplementation to apoE-deficient mice. Similarly, there was no change in this biomarker after the intake of concentrated grape juice[80] or grape seed polyphenols[46] by human subjects. Once again, these differences may be due to the different doses employed or to the phenolic composition of the tested products.

In contrast, biomarkers of protein and DNA oxidation have proven to be useful tools for evaluating the effect of supplementation with grape-derived products, and although not many studies have used them, the results tend to agree. A decrease in protein oxidation has been observed after polyphenol-enriched red wine and white wine supplementation to hamsters with an atherogenic diet[61] and after concentrated grape juice supplementation to human subjects[80]. As regards DNA, a decrease in damage to neuronal DNA was observed after supplementing gerbils with freeze-dried grapes[102]. In trials in human subjects, there was a decrease in the 8-oxo-deoxygunaosin:deoxiguanosin ratio after the intake of procyanidins from grape seeds, sustained for 1 week after cessation of the treatment[98], and after supplementation with red wine to patients with acute coronary syndrome[103].

Measurement of antioxidant compounds

Several studies have dealt with the evolution of plasma polyphenols after the intake of grapes and derived products. The bioavailability of dietary polyphenols has been reviewed elsewhere[104,105], so we will only refer here to observations after chronic and not after acute intakes, which have tended to produce positive results. Plasma polyphenols increased after the intake of red wine, polyphenol-enriched white wine or red wine polyphenols[15,21,88]. Similarly, an increase was observed in the urinary excretion of 4-o-methyl-gallic acid after the consumption of red wine and dealcoholised red wine[67], in the urinary excretion of four metabolites from flavan-3-ols after the intake of red wine[77] and in the urinary excretion of resveratrol metabolites after the intake of red and white wine[17].

Vitamins C and E are plasma constituents that exhibit antioxidant capacity and whose plasma concentrations may be affected by polyphenol metabolism. Because of this, some intervention studies with antioxidants have evaluated the evolution of the concentration of these compounds.

In studies in animals, the intake of red wine and ethanol caused a significant increase in plasma vitamin C[62] in rats, and concentrated grape pomace supplementation increased liver vitamin E[95] in chickens, while dealcoholised red wine supplementation did not modify plasma levels of vitamins C and E in apoE-deficient hamsters[86].

In the case of human subjects, no modification in plasma antioxidant vitamin concentrations has been observed after the intake of white wine, red wine, dealcoholised red wine or grape procyanidins[25,67,98]. However, some interesting observations regarding vitamin E should be noted: the intake of red wine polyphenols increased vitamin E content in LDL by 15 %[32]; the intake of grape procyanidins did not modify vitamin E levels in plasma, but did increase them in erythrocytes[98]; although there was no increase in plasma vitamin C after the intake of concentrated grape juice, when its concentration was normalised dividing it between plasma cholesterol, there was a significant increase due to the treatment[65]. These data show that grape polyphenols do not have the same effect on the plasma levels of vitamin C and of vitamin E, and also that in the latter case polyphenols would contribute to its regeneration, as noted earlier[106].

Hepatic enzymes

Aerobic organisms possess enzymic systems that counteract the effect of free radicals. The activity of these systems may be reduced as a consequence of stress situations, and increased after the intake of polyphenol-rich products. Some trials on animals to determine the effects of grapes and derived products have measured the activity of these enzymes.

Roig et al.[101] observed an increase in the activities of superoxide dismutase and glutathione peroxidase in rats 45 d after supplementation with red wine; however, this was not sustained over 6 months of intake, at the end of which a reduction was observed in the levels of MDA in plasma and other tissues. The authors concluded that the intake of red wine initially activated antioxidant enzymes, but the long-term effect was to reduce lipid peroxidation. Similarly, red wine and ethanol supplementation caused an increase in the activities of glutathione peroxidase in the renal cortex and renal papilla of rats[93]. Also, an increase in hepatic glutathione peroxidase activity after the intake of grape pomace by rats was observed, although there was no change in any of the other hepatic enzymes, and there was a significant decrease in the activities of these enzymes in the

erythrocytes. A similar tendency was observed for tomato and apple pomace[26].

Effects on glycaemia

In a trial in fructose-fed rats, a model of insulin resistance without obesity, when the rats were supplemented with grape polyphenols, there was no change in plasma glucose or insulin, but there was a significant reduction in the homeostasis model assessment of insulin resistance (HOMA:ir) index, which measures insulin resistance[43].

As regards studies in human subjects, some trials have produced positive results. In those that reported no effect, this may be related to the design of the study. Williams et al. [20] observed a decrease in plasma glucose 6 h after intake of red wine or white wine by coronary artery disease patients, and Gin et al. [107] reported that maximum glucose excursion after an acute intake of red wine or tannic acid (phenolic present in red wine) was significantly lower than after the intake of water or ethanol alone.

Two trials reported no effect on plasma glucose, insulin, insulin sensitivity or HOMA:ir after supplementation with red wine or dealcoholised red wine[66,69]. However, one of these studies was performed in moderately obese women[66]; the authors discussed the possibility of an interaction between the alcohol dose and the body composition that could have masked potential beneficial changes in insulin sensitivity, since it is known that the metabolic response of overweight subjects, in terms of glycaemia and insulin sensitivity, differs from the response of normal-weight subjects[107].

Other authors have suggested that, although polyphenols do not have a direct effect on postprandial glycaemia, they may affect other related parameters. For instance, Ceriello et al. [100] found no difference in plasma glucose in type 2 diabetic patients after a meal accompanied by red wine as compared with a control group. Nevertheless, this study found that the intake of wine had a beneficial effect on postprandial oxidative stress, a key step in the generation of free radicals in diabetic patients[98].

Conclusions

From the in vivo studies performed during the last 13 years addressing the effects of grape products on different parameters related to CVD that were reviewed, the following effects have been observed:

(1) A hypotensive effect, observed in several studies in animals and in human subjects, mainly due to an increase in the release of NO.

(2) A hypolipidaemic effect, reducing levels of plasma total cholesterol, LDL-cholesterol and TAG. The results in human subjects suggest that these effects are more pronounced the higher the plasma lipids at baseline are. The hypolipidaemic effect of polyphenols would be related to the fact that these compounds may absorb cholesterol, bile acids and other dietary lipids and increase their faecal excretion.

(3) An anti-atherosclerotic effect in the early stages of development of atherosclerosis, observed as a reduction in atheromatous plaque and in LDL oxidation. This effect appears to be due to the inhibition of cell proliferation in smooth muscle and to antioxidant protection of LDL.

(4) An improvement in antioxidant status measured in terms of plasma antioxidant capacity, oxidation biomarkers, antioxidant compounds and antioxidant enzymes.

The presence of dietary fibre in some of the tested products may enhance some of the above effects.

Differences in the design of the studies and in the composition of the tested products (not always provided) can explain the different results produced by some of these assays. The relative contribution of major constituents such as polyphenols, ethanol and dietary fibre remains to be elucidated.

References

1 Castelli WP (1996) Lipids, risk factors and ischaemic heart disease. Atherosclerosis 124, Suppl., S1–S9.

2 Wilson PW, D'Agostino RB, Levy D, et al. (1998) Prediction of coronary heart disease using risk factor categories. Circulation 97, 1837–1847.

3 Panagiotakos DB, Pitsavos C, Arvaniti F, et al. (2007) Adherence to the Mediterranean food pattern predicts the prevalence of hypertension, hypercholesterolemia, diabetes and obesity, among healthy adults; the accuracy of the MedDietScore. Prev Med 44, 335–340.

4 Arts IC & Hollman PC (2005) Polyphenols and disease risk in epidemiologic studies. Am J Clin Nutr 78, Suppl. 1, 559S–569S.

5 Renaud S & De Lorgeril M (1992) Wine, alcohol, platelets and the French paradox for coronary heart disease. Lancet 339, 1523–1526.

6 Bravo L (1998) Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. Nutr Rev 56, 317–333.

7 Leighton F & Urquiaga I (2007) Changes in cardiovascular risk factors associated with wine consumption in intervention studies in humans. Ann Epidemiol 17, S32–S36.

8 Pérez-Vizcaíno F, Duarte J & Andriantsitohaina R (2006) Endothelial function and cardiovascular disease: effects of quercetin and wine polyphenols. Free Rad Res 40, 1054–1065.

9 Girar B & Mazza G (1998) Functional grape and citrus products. In Functional Foods. Biochemical and Processing Aspects, pp. 139–192 [G Mazza, editor]. Lancaster, PA: Technomic Publishing Company.

10 Cui J, Cordis GA, Tosaki A, et al. (2002) Reduction of myocardial ischemia reperfusion injury with regular consumption of grapes. New York Acad Sci 957, 302–307.

11 Zern TL, West KL & Fernández ML (2003) Grape polyphenols decrease plasma triglycerides and cholesterol

accumulation in aorta of ovariectomized guinea pigs. *J Nutr* **133**, 2268–2273.

12. Ruf JC, Berger JL & Renaud S (1995) Platelet rebound effect of alcohol withdrawal and wine drinking in rats. Relation to tannins and lipid peroxidation. *Arterioscler Thromb Vasc Biol* **15**, 140–144.

13. Martín-Carrón N, Saura-Calixto F & Goñi I (2000) Effects of dietary fibre and polyphenol-rich grape products on lipidaemia and nutritional parameters in rats. *J Sci Food Agric* **80**, 1183–1188.

14. Falchi M, Bertelli A, Lo Scalzo R, *et al.* (2006) Comparison of cardioprotective abilities between the flesh and skin of grapes. *J Agric Food Chem* **54**, 6613–6622.

15. Shahidi F & Naczk M (2004) *Phenolics in Foods and Nutraceuticals*. Boca Raton, FL: CRC Press.

16. Badía E, Sacanella E, Fernández-Solá J, *et al.* (2004) Decreased tumor necrosis factor-induced adhesion of human monocytes to endothelial cells after moderate alcohol consumption. *Am J Clin Nutr* **80**, 225–230.

17. Sacanella E, Vázquez-Agell M, Mena MP, *et al.* (2007) Down-regulation of adhesion molecules and other inflammatory biomarkers after moderate wine consumption in healthy women: a randomized trial. *Am J Clin Nutr* **86**, 1463–1469.

18. Agewall S, Wright S, Doughty RN, *et al.* (2000) Does a glass of red wine improve endothelial function? *Eur Heart J* **21**, 74–78.

19. Naissides M, Mamo JCL, James AP, *et al.* (2004) The effect of acute red wine polyphenol consumption on postprandial lipaemia in postmenopausal women. *Atherosclerosis* **177**, 401–408.

20. Williams MJA, Sutherland WHF, Whelan AP, *et al.* (2004) Acute effect of drinking red and white wines on circulating levels of inflammation-sensitive molecules in men with coronary artery disease. *Metabolism* **53**, 318–323.

21. Pignatelli P, Ghiselli A, Buchetti B, *et al.* (2006) Polyphenols synergistically inhibit oxidative stress in subjects given red and white wine. *Atherosclerosis* **188**, 77–83.

22. Osman HE, Maalej N, Shanmunagayagam D, *et al.* (1998) Grape juice, but not orange or grapefruit juice inhibits platelet activity in dogs and monkeys (*Macaca fasciularis*). *J Nutr* **128**, 2307–2312.

23. Shanmuganayagam D, Warner TF, Krueger CG, *et al.* (2007) Concord grape juice attenuates platelet aggregation, serum cholesterol and development of atheroma in hypercholesterolemic rabbits. *Atherosclerosis* **190**, 135–142.

24. Castilla P, Dávalos A, Teruel JL, *et al.* (2008) Comparative effects of dietary supplementation with grape juice and vitamin E on production of superoxide by circulating neutrophil NADPH oxidase in hemodialysis patients. *Am J Clin Nutr* **87**, 1053–1061.

25. De Rijke YB, Demacker PMN, Assen NA, *et al.* (1996) Red wine consumption does not affect oxidizability of low-density lipoproteins in volunteers. *Am J Clin Nutr* **63**, 329–334.

26. Bobek P, Ozdin L & Hromadova M (1998) The effect of dried tomato, grape and apple pomace on the cholesterol metabolism and antioxidative enzymatic system in rats with hypercholesterolemia. *Nahrung* **42**, 317–320.

27. Martin-Carrón N, Goñi I, Larrauri JA, *et al.* (1999) Reduction in serum total and LDL cholesterol concentrations by a dietary fiber and polyphenol-rich grape product in hypercholesterolemic rats. *Nutr Res* **19**, 1371–1381.

28. Bravo L & Saura-Calixto F (1998) Characterization of dietary fiber and the *in vitro* indigestible fraction of grape pomace. *Am J Enol Vitic* **49**, 135–141.

29. Pérez-Jiménez J, Serrano J, Tabernero M, *et al.* (2008) Effects of grape antioxidant dietary fiber on cardiovascular disease risk factors. *Nutrition* **24**, 646–653.

30. Monagas M, Hernández-Ledesma B, Gómez-Cordovés C, *et al.* (2006) Commercial dietary ingredients from *Vitis vinifera* L. leaves and grapes skins: antioxidant and chemical chracterization. *J Agric Food Chem* **54**, 319–327.

31. Monagas M, Hernández-Ledesma B, Garrido I, *et al.* (2005) Quality assessment of commercial dietary antioxidant products from *Vitis vinifera* L. grape seeds. *Nutr Cancer* **53**, 244–254.

32. Carbonneau MA, Leger CL, Monnier L, *et al.* (1997) Supplementation with wine phenolic compounds increases the antioxidant capacity of plasma and vitamin E of low-density lipoprotein without changing the lipoprotein Cu^{2+}-oxidizability: possible explanation by phenolic location. *Eur J Clin Nutr* **51**, 682–690.

33. Vinson JA, Proch J & Bose P (2001) Mega Natural® Gold Grapeseed Extract: *in vitro* antioxidant and *in vivo* human supplementation studies. *J Med Food* **4**, 17–25.

34. Goñi I, Martín N & Saura-Calixto F (2005) *In vitro* digestibility and intestinal fermentation of grape seed and peel. *Food Chem* **90**, 281–286.

35. National Institutes of Health (1997) *The Sixth Report of Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure*. Bethesda, MD: National Institutes of Health, NIH Publication no. 98–4080.

36. Puddey IB, Beilin LJ, Vandogen R, *et al.* (1987) Regular alcohol use raises blood pressure in treated hypertensive subjects. A randomised controlled trial. *Lancet* **i**, 647–651.

37. Klatsky AL, Friedman GD & Armstrong MA (1986) The relationship between alcoholic beverage and use of other traits to blood pressure: a new Kaiser Permanet study. *Circulation* **73**, 628–636.

38. Bulpitt CJ, Shipley MJ & Semmence A (1987) The contribution of a moderate intake of alcohol to the presence of hypertension. *J Hypertens* **5**, 85–91.

39. Zilkens RR, Burke V, Hodgson JM, *et al.* (2005) Red wine and beer elevate blood pressure in normotensive men. *Hypertension* **45**, 874–879.

40. Diebolt M, Bucher B & Andriantsitohaina R (2001) Wine polyphenols decrease blood pressure, improve NO vasodilatation and induce gene expression. *Hypertension* **38**, 159–165.

41. Bernatova I, Pechanova O, Babal P, *et al.* (2002) Wine polyphenols improve cardiovascular remodelling and vascular function in NO-deficient hypertension. *Am J Physiol Heart Circ Physiol* **282**, H942–H948.

42. Soares de Moura R, Costa Viana FS, Souza MAU, *et al.* (2002) Antihypertensive, vasodilator and antioxidant effects of a vinifera grape skin extract. *J Pharm Pharmacol* **54**, 1515–1520.

43. Al-Awwadi NA, Bournet A, Azay J, *et al.* (2004) Red wine polyphenols alone or in association with ethanol prevent hypertension, cardiac hyperthrophy and production of reactive oxygen species in the insulin-resistant fructose-fed rats. *J Agric Food Chem* **52**, 5593–5597.

44. Ranaivo HR, Diebolt M & Andriantsitohaina R (2004) Wine polyphenols induce hypotension and decrease cardiac reactivity and infarct size in rats: involvement of nitric oxide. *Br J Pharmacol* **142**, 671–678.

45. Park YK, Kim JS & Kang MY (2004) Concord grape juice supplementation reduces blood pressure in Korean hypertensive men: double-blind, placebo controlled intervention trial. *Biofactors* **22**, 145–147.

46. Ward NC, Hodgson JM, Croft KD, *et al.* (2005) The combination of vitamin C and grape-seed polyphenols

increases blood pressure: a randomized, double-blind, placebo-controlled trial. *J Hypertens* **23**, 427–434.

47 Chou EJ, Keevil JG, Aeschlimann S, *et al*. (2001) Effect of ingestion of purple grape juice on endothelial function in patients with coronary artery disease. *Am J Cardiol* **88**, 553–555.

48 Wallerath T, Poleo D, Li H, *et al*. (2003) Red wine increases the expression on human endothelial nitric oxide synthase. *J Am Coll Cardiol* **41**, 471–478.

49 Andriambeloson E, Kleschyov AL, Muller B, *et al*. (1997) Nitric oxide production and endothelium-dependent vasor-elaxation induced by wine polyphenols in rat aorta. *Br J Pharmacol* **120**, 1053–1058.

50 Wollny T, Aiello L, Di Tommaso D, *et al*. (1999) Modulation of haemostatic function and prevention of experimental thrombosis by red wine in rats: a role for increased nitric oxide production. *Br J Pharmacol* **127**, 747–755.

51 Matsuo S, Nakamura Y, Takahashi M, *et al*. (2001) Effect of red wine and ethanol on production of nitric oxide in healthy subjects. *Am J Cardiol* **87**, 1029–1031.

52 Freedman JE, Parker C III, Li L, *et al*. (2001) Select flavonoids and whole juice from purple grapes inhibit platelet function and enhance nitric oxide release. *Circulation* **103**, 2792–2798.

53 Boban M, Modun D, Musc I, *et al*. (2006) Red wine induced modulation of vascular function: separating the role of polyphenols, ethanol and urates. *J Cardiovasc Pharmacol* **47**, 695–701.

54 Hashimoto M, Kim S, Eto M, *et al*. (2001) Effect of intake of red wine on flow-mediated vasodilation of the brachial artery. *Am J Cardiol* **88**, 1457–1460.

55 Lekakis J, Rallidis LS, Andreadou I, *et al*. (2005) Polyphenolic compounds from red grapes acutely improve endothelial function in patients with coronary heart disease. *Eur J Cardiovasc Prev Rehabil* **12**, 596–600.

56 Stein JH, Keevil JG, Wiebe DA, *et al*. (1999) Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation* **100**, 1050–1055.

57 Clifton PM (2004) Effect of grape seed extracts and quercetin on cardiovascular and endothelial parameters in high-risk subjects. *J Biomed Biotech* **2004**, 272–278.

58 Vinson JA, Teufel K & Wu N (2001) Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in a hamster model. *Atherosclerosis* **156**, 67–72.

59 Auger C, Caporiccio B, Landrault C, *et al*. (2002) Red wine phenolic compounds reduce plasma lipids and apolipopro-tein B and prevent early aortic atherosclerosis in hypercholesterolemic Golden Syrian hamsters (*Mesocrice-tus auratus*). *J Nutr* **132**, 1207–1213.

60 Auger C, Gerain P, Laurent-Bichon F, *et al*. (2004) Phenolics from commercialized grape extracts prevent early athero-sclerotic lesions in hamsters by mechanisms other than antioxidant effect. *J Agric Food Chem* **52**, 5297–5302.

61 Auger C, Rouanet JM, Vanderlinde R, *et al*. (2005) Polyphenols-enriched Chardonnay white wine and sparkling pinot noir red wine identically prevent early atherosclerosis in hamsters. *J Agric Food Chem* **53**, 9823–9829.

62 Cestaro B, Simonetti P, Cervato G, *et al*. (1996) Red wine effects on peroxidation indexes of rat plasma and erythrocytes. *Int J Food Sci Nutr* **47**, 181–189.

63 Yamakoshi J, Kataoka S, Koga T, *et al*. (1999) Proantho-cyanidin-rich extract from grape seeds attenuate the development of aortic atherosclerosis in cholesterol-fed rabbits. *Atherosclerosis* **142**, 139–149.

64 Araya J, Rodrigo R, Orellana M, *et al*. (2001) Red wine raises plasma HDL and preserves long-chain polyunsatu-rated fatty acids in rat kidney and erythrocytes. *Br J Nutr* **86**, 189–195.

65 Castilla P, Echarri R, Davalos A, *et al*. (2006) Concentrated red grape juice exerts antioxidant hypolipidemic and antiinflamatory effects in both hemodyalisis patients and healthy subjects. *Am J Clin Nutr* **84**, 252–262.

66 Cordain L, Melby C, Hamamoto AE, *et al*. (2000) Influence of moderate chronic wine consumption on insulin sensitivity and other correlates of syndrome X in moderately obese women. *Metabolism* **49**, 1473–1478.

67 Cacetta RZZ, Burke V, Mori TA, *et al*. (2001) Red wine polyphenols, in the absence of alcohol, reduce lipid peroxidative stress in smoking subjects. *Free Rad Biol Med* **30**, 636–642.

68 Zern TL, Wood RS, Greene C, *et al*. (2005) Grape polyphenols exert a cardioprotective effect in pre- and post-menopausal women by lowering plasma lipids and reducing oxidative stress. *J Nutr* **135**, 1911–1917.

69 Naissides M, Mamo JCL, James AP, *et al*. (2006) The effect of chronic consumption of red wine on cardiovascular disease risk factors in postmenopausal women. *Athero-sclerosis* **185**, 438–445.

70 Hayek T, Fuhrman B, Vaya J, *et al*. (1997) Reduced progression of atherosclerosis in apolipoprotein E-deficient mice following consumption of red wine or its polyphenols quercetin or catechin, is associated with reduced suscepti-bility of LDL to oxidation and aggregation. *Arterioscler Thromb Vasc Biol* **17**, 2744–2752.

71 Tebib K, Besancon P & Rouanet JM (1994) Dietary grape seed tannins affect lipoproteins, lipoprotein lipases and tissue lipids in rats fed hypercholesterolemic diets. *J Nutr* **124**, 2451–2457.

72 Nakamura Y & Tonogai Y (2002) Effects of grape seed polyphenols on serum and hepatic lipid contents and fecal steroid excretion in normal and hypercholesterolemic rats. *J Health Sci* **48**, 570–578.

73 Pal S, Naissides M & Mamo J (2004) Polyphenolics and fat absorption. *Int J Obes Related Metab Disord* **28**, 324–326.

74 Ikeda I, Imasato Y, Sasaki E, *et al*. (1992) Tea catechins decrease micellar solubility and intestinal absorption of cholesterol in rats. *Biochim Biophys Acta* **1127**, 141–146.

75 Fuhrman B, Lavy A & Aviram M (1995) Consumption of red wine with meals reduces the susceptibility of human plasma and low-density lipoproteins to lipid peroxidation. *Am J Clin Nutr* **61**, 549–554.

76 Hansen AS, Marckmann P, Dragsted LO, *et al*. (2005) Effect of red wine and red grape extract on blood lipids, haemostatic factors and other risk factors for cardiovascular disease. *Eur J Clin Nutr* **59**, 449–455.

77 Tsang C, Higgings S, Duthie GG, *et al*. (2005) The influence of moderate red wine consumption on antioxidant status and indices of oxidative stress associated with CHD in healthy volunteers. *Br J Nutr* **93**, 233–240.

78 Avellone G, Di Garbo V, Campisi D, *et al*. (2006) Effects of moderate Sicilian red wine consumption on inflammatory biomarkers of atherosclerosis. *Eur J Clin Nutr* **60**, 41–47.

79 Ayaori M, Ishikawa T, Yoshida M, *et al*. (1997) Beneficial effects of alcoholic withdrawal on LDL particle size distribution and oxidative susceptibility in subjects with alcohol-induced hypertriglyceridemia. *Arterioscler Thromb Vasc Biol* **17**, 2540–2547.

80 O'Byrne DJ, Devaraj S, Grundy SM, *et al*. (2002) Comparison of the antioxidant effects of Concord grape juice flavonoids and α-tocopherol on markers of oxidative stress in healthy adults. *Am J Clin Nutr* **76**, 1367–1374.

81 Keevil JG, Osman HE, Reed JD, *et al.* (2000) Grape juice, but nor orange juice or grapefruit juice, inhibits human platelet aggregation. *J Nutr* **130**, 53–56.

82 Pace-Asciak CR, Rounova O, Hahn SE, *et al.* (1996) Wines and grape juices as modulators of platelet aggregation in healthy human subjects. *Clin Chim Acta* **246**, 163–182.

83 Frederiksen S, Mortensen A, Schrøder M, *et al.* (2007) Effect of red grape skin and seed extract supplementation on atherosclerosis in Watanabe heritable hyperlipidemic rabbits. *Molec Nutr Food Res* **251**, 564–571.

84 Waddington E, Puddey IB & Croft KD (2004) Red wine polyphenolic compounds inhibit atherosclerosis in apolipoprotein E-deficient mice independently of effects on lipid peroxidation. *Am J Clin Nutr* **79**, 54–61.

85 Benteon JF, Skovenborg E, Hansen C, *et al.* (2001) Red wine does not reduce mature atherosclerosis in apolipoprotein E deficient mice. *Circulation* **103**, 1681–1687.

86 Stocker R & O'Halloran RA (2004) Dealcoholized red wine decreases atherosclerosis in apolipoprotein E gene-deficient mice independently of inhibition of lipid peroxidation in the artery wall. *Am J Clin Nutr* **79**, 123–130.

87 Xia J, Allenbrand B & Sun GY (1998) Dietary supplementation of grape polyphenols and chronic ethanol administration on LDL oxidation and platelet function in rats. *Life Sci* **63**, 383–390.

88 Nidgikar SV, Williams NR, Griffin BA, *et al.* (1998) Consumption of red wine polyphenols reduces the susceptibility of low-density lipoproteins to oxidation *in vivo*. *Am J Clin Nutr* **68**, 258–265.

89 Estruch R, Sacanella E, Badia E, Antunez E, *et al.* (2004) Different effects of red wine and gin consumption on inflammatory biomarkers of atherosclerosis: a prospective randomised crossover trial. Effects of wine on inflammatory markers. *Atherosclerosis* **175**, 117–123.

90 Ducros V, Demuth K, Sauvant MP, *et al.* (2002) Methods for homocysteine analysis and biological relevance of the results. *J Cromatogr B Analyt Technol Biomed Life Sci* **781**, 207–226.

91 King DE (2005) Dietary fiber, inflammation and cardio-vascular disease. *Molec Nutr Food Res* **49**, 594–600.

92 Van der Gaag MS, Ubbink JB, Sillanaukee P, *et al.* (2000) Effect of consumption of red wine, spirits and beer on serum homocysteine. *Lancet* **355**, 1522.

93 Rodrigo R, Rivera G, Orellana M, *et al.* (2002) Rat kidney antioxidant response to long-term exposure to flavonol rich red wine. *Life Sci* **71**, 2281–2295.

94 Goñi I & Serrano J (2005) The intake of dietary fiber from grape seeds modifies the antioxidant status in rat cecum. *J Sci Food Agric* **85**, 1877–1881.

95 Goñi I, Brenes A, Centeno C, *et al.* (2007) Effect of dietary grape pomace and vitamin E on growth performance, nutrient digestibility, and susceptibility to meat lipid oxidation in chickens. *Poult Sci* **86**, 508–516.

96 Brenes A, Viveros A, Goñi I, *et al.* (2008) Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. *Poult Sci* **87**, 307–316.

97 Wirleitner B, Schröecksnadel K, Winkler C, *et al.* (2003) *In vivo* determination of oxidative stress. *Aktuel Ermaehr Med* **28**, 363–370.

98 Simonetti P, Ciapellano S, Gardana C, *et al.* (2002) Procyanidins from *Vitis vinifera* seeds: *in vivo* effects on oxidative stress. *J Agric Food Chem* **50**, 6217–6221.

99 Pérez-Jiménez J (2007) Metodología para la evaluación de ingredientes funcionales antioxidantes. Efecto de fibra antioxidante de uva en status antioxidante y parámetros de riesgo cardiovascular en humanos (Methodology for the evaluation of functional antioxidant ingredients. Effect of grape antioxidant dietary fibre on antioxidant status and cardiovascular risk in humans). PhD Thesis. Department of Applied Physical Chemistry, Universidad Autónoma de Madrid, Spain.

100 Ceriello A, Bortolotti N, Motz E, *et al.* (2001) Red wine protects diabetic patients from meal-induced oxidative stress and thrombosis activation: a pleasant approach to the prevention of cardiovascular disease in diabetes. *Eur J Clin Invest* **31**, 322–328.

101 Roig R, Cascón E, Arola L, *et al.* (1999) Moderate red wine consumption protects the rat against oxidation *in vivo*. *Life Sci* **17**, 1517–1524.

102 Wang Q, Simona A, Li W, *et al.* (2005) Dietary grape supplement ameliorates cerebral ischemia-induced neuronal death in gerbils. *Molec Nutr Food Res* **49**, 443–451.

103 Guarda E, Godoy I, Foncea R, *et al.* (2005) Red wine reduces oxidative stress in patients with acute coronary syndrome. *Int J Cardiol* **104**, 35–38.

104 Manach C, Williamson G, Morand C, *et al.* (2005) Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr* **81**, Suppl. 1, 230S–242S.

105 Williamson G & Manach C (2005) Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *Am J Clin Nutr* **81**, Suppl. 1, 243S–255S.

106 Zhou B, Wu LM, Yang L, *et al.* (2005) Evidence for a α-tocopherol regenerating reaction of green tea polyphenols in SDS micelles. *Free Rad Biol Med* **38**, 78–84.

107 Gin H, Rigalleau V, Caubet O, *et al.* (1999) Effects of red wine, tannic acid or ethanol on glucose tolerance in non-insulin-dependent diabetic patients and on starch digest-ibility *in vitro*. *Metabolism* **48**, 1179–1183.

108 Whelan AP, Sutherland WHF, McCormick MP, *et al.* (2004) Effects of white and red wine on endothelial function ins subjects with coronary artery disease. *Int Med J* **34**, 224–228.

Nutrition Research Reviews

NS