Short Communication

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

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Abstract

Moderate wine consumption has been shown to lower cardiovascular risk. One of the mechanisms could involve the control of postprandial hyperlipaemia, a well-defined risk factor for atherosclerosis, reasonably by reducing the absorption of lipid oxidised species from the meal. The objective of the present study was to investigate whether wine consumption with the meal is able to reduce the postprandial increase in plasma lipid hydroperoxides and cholesterol oxidation products, in human subjects. In two different study sessions, twelve healthy volunteers consumed the same test meal rich in oxidised and oxidisable lipids (a double cheeseburger), with 300 ml of water (control) or with 300 ml of red wine (wine). The postprandial plasma concentration of cholesterol oxidation products was measured by GC–MS. The control meal induced a significant increase in the plasma concentration of lipid hydroperoxides and of two cholesterol oxidation products, 7β-hydroxycholesterol and 7-ketocholesterol. The postprandial increase in lipid hydroperoxides and cholesterol oxidation products was fully prevented by wine when consumed with the meal. In conclusion, the present study provides evidence that consumption of wine with the meal could prevent the postprandial increase in plasma cholesterol oxidation products.

Key words: Red wine; Postprandial oxidative stress; Oxycholesterols; Human studies

Epidemiological studies have indicated that wine can be considered protective against CVD development when its moderate consumption is inserted in a correct lifestyle(1), including the ‘instructions to drink/use’, i.e. ‘to be taken with meals’. A number of experimental studies have suggested that red wine compounds, especially polyphenols, might play a role in preventing the development and progression of atherosclerosis, acting through different pathways that include inhibition of lipid peroxidation, metal chelation, free-radical scavenging, inhibition of platelet aggregation, anti-inflammatory and oestrogenic activity, improvement of endothelial function, lowering of blood pressure and modulation of lipoprotein metabolism(2). The attenuation of postprandial oxidative stress could be one of the mechanisms explaining the protective action of wine phenols(3,4). In fact, the absorption of pro-oxidant/oxidised species with the meal can induce physiological events, such as the formation of mildly oxidised lipoprotein(5) or endothelial dysfunction(6), and inflammatory responses(7), all events linked to the development of CVD.

There is evidence that oxycholesterols are angiototoxic and could cause atherosclerosis(8). Animal studies have shown that the addition of oxidised cholesterol to the diet increases atherosclerosis(9), and epidemiological studies have shown an association between plasma oxycholesterols and CVD(10). Oxycholesterols have also shown to possess mutagenic and carcinogenic effects in both in vivo and in vitro studies(11).

The typical Western diet contains substantial quantities of oxidised cholesterol, and the mean dietary intake has been estimated in mg/d per person(12).

In view of the health implications of oxycholesterol absorption from food, we investigated, in a pilot study, the possibility that wine consumption with a meal influences the postprandial increase in plasma lipid hydroperoxides and oxycholesterols in humans.
**Oxycholesterol measurement**

The following four different oxycholesterols were measured in both the meal and plasma: 7-ketocholesterol (7-Keto-C), 5α,6α-epoxycholesterol, 5β,6β-epoxycholesterol and 7β-hydroxycholesterol by GC–MS.\(^{17}\)

The four oxycholesterols were selected because they are the most abundant in food and efficiently absorbed.\(^{18}\)

Briefly, plasma samples (200 µl) were added with 1 µg of the internal standard (19-hydroxycholesterol). Saponification was carried out under N\(_2\) flow at 60°C for 90 min with 1 ml of 1 M-NaOH ethanolic solution. Samples were then extracted with cyclohexane, and the resulting organic layer was evaporated to dryness under N\(_2\). Then, they were resuspended in 1 ml hexane and applied to solid-phase extraction (Supelclean LC–Si cartridge; Sigma).\(^{19}\) The oxycholesterol fraction was dried under N\(_2\) and derivatised with 70 µl of the Sylon BTZ kit (at room temperature for 45 min). GC–MS analyses were performed on an Agilent 6890A gas chromatograph coupled to a 5973N quadrupole mass-selective detector (Agilent Technologies, Palo Alto, CA, USA). Gas chromatographic separations were carried out on an Agilent HP-5MS fused silica capillary column (inner diameter 30 m × 0.25 mm and film thickness 0.25 µm). The injection mode was splitless at a temperature of 280°C. The column temperature program was as follows: 160°C (1 min) to 280°C at a rate of 20°C/min and held for 15 min. The carrier gas was He at a constant flow of 1.0 ml/min. The spectra were obtained in electron impact mode at 70 eV ionisation energy; ion source temperature was 280°C and ion source vacuum was 10\(^{-5}\) Torr (1.3 × 10\(^{-3}\) Pa). Analyses were performed both in total ion current and selected-ion monitoring modes. Selected-ion monitoring analyses were carried out by selecting the following representative ions: \(m/z\) 355 for the 19-0H-C trimethylsilyl (TMS) derivative; \(m/z\) 456 for the 7β-hydroxycholesterol TMS derivative; \(m/z\) 474 for the 5β,6β-epoxycholesterol TMS derivative; \(m/z\) 474 for the 5α,6α-epoxycholesterol TMS derivative; \(m/z\) 472 for the 7-Keto-C TMS derivative.

**Statistical analysis**

Data are presented as means and standard deviations. Statistical analysis was carried out using repeated-measures ANOVA, followed by Tukey’s test for multiple comparisons. Analyses were performed with KaleidaGraph software (version 3.6; Synergy Software, Reading, PA, USA). Values <0.05 were considered statistically significant.

**Results**

**Wine composition**

Total polyphenols (1871 mg/l, as catechin equivalents) were in the typical range for the variety. The concentration of total proanthocyanidins was 167.7 mg/l. The total administered dose of the major phenolics was calculated from the concentration in wine measured by HPLC at the time of the experiment. The wine had a quite high content of free anthocyanins, and the total administered dose was of 304.1 µmol. Hydroxycinnamates (85.2 µmol administered) consisted mainly of trans-caftaric acid, coumaric acid and trans-coumaric acid, with minor amounts of ferric acid and grape reaction product (i.e. trans-2S-glutathionyl-coumaric acid). Free flavanols (total of 82.9 µmol) consisted of epigallocatechin, (+)-catechin, epicatechin and gallo catechin. Myricetin was by far the main flavonol (20.5 µmol of total flavonols). Other minor phenolics were tyrosol (29.8 µmol) and the four monomers of resveratrol (for a total of 5.4 µmol).

In summary, the single dose of Teroldego wine provided 561 mg of phenolics (which is approximately in the millimolar level, assuming an average molecular weight of 500).
Lipid hydroperoxides and oxycholesterols in the test meal

The lipid hydroperoxide content of the test meal was 237 (SD 36) μmol of H₂O₂ equivalents.

As for oxycholesterols, the test meal contained 498 (SD 147) μg of 7-ketocholesterol, 138 (SD 48) μg of 5α,6α-epoxycholesterol, 91 (SD 6) μg of 7β-hydroxycholesterol and 70 (SD 6) μg of 5β,6β-epoxycholesterol.

When expressed per g of the test meal, total oxycholesterols were 3.9 (SD 1.1) μg/g, and this value is in accordance with literature data. van de Bovenkamp et al. (20) reported 3.6 μg of total oxycholesterols/g of a cooked mixed Dutch diet, while Baggio et al. (20) and Rodriguez-Estrada et al. (21) reported a concentration of about 2 μg/g of hamburger.

Effect of the control and wine meals on plasma lipids, lipid hydroperoxides and oxycholesterols

Plasma concentrations of total cholesterol, TAG and alcohol, before and after the control and wine meals, are shown in Table 1. As expected, there was an increase in plasma TAG after the consumption of both meals, while ethanol, as expected, increased significantly only after the wine meal. Cholesterol concentration did not change significantly after both meals.

As shown in Fig. 1, the control meal induced a significant increase in total plasma lipid hydroperoxides. On the contrary, the wine meal not only prevented this increase, but also reverted it, inducing a significant decrease in plasma lipid hydroperoxides.

Fig. 1 shows also the effect of the meal on plasma oxycholesterols. The control meal induced a significant increase in 7β-hydroxycholesterol and 7-ketocholesterol concentrations. This increase was statistically significant 1 h after the consumption of the meal. The postprandial increase in these two oxycholesterols was fully prevented when wine was consumed with the meal. Indeed, wine consumption induced a significant decrease in 7β-hydroxycholesterol. Both 5α,6α-epoxycholesterol and 5β,6β-epoxycholesterol showed the same trend as observed for 7β-hydroxycholesterol and 7-ketocholesterol, even if their postprandial changes (after both meals) were not statistically significant.

An estimation based on the subjects’ volume of plasma (55% of volume of blood, calculated individually as 7% of their body weight (22)) indicates that total plasma oxycholesterols (the sum of the measured four oxycholesterols) represented 105 (SD 29) and 95 (SD 26)% of the ingested dose 1 and 3 h after the control meal, respectively. As evident from Fig. 1, after the wine meal, total plasma oxycholesterols decreased below the baseline value (– 55 (SD 28) and – 31 (SD 41)% of the ingested dose, at 1 and 3 h, respectively).

Discussion

Some authors suggest that the absorption from meals of the products of lipid oxidation could be, at least partially, the link between postprandial lypaemia and atherosclerosis (23).

Oxycholesterols are a common component of the Western diet, and their presence is striking in fast food and processed food. Studies in both humans (18) and animals (24) have demonstrated that oxycholesterols are absorbed by the small intestine, transported in plasma by chylomicrons and incorporated into lipoprotein. As oxycholesterols possess several proatherogenic activities (8), a delayed clearance of these compounds from the circulation could be harmful.

Although oxysterols are principally derived from dietary sources, circulating oxycholesterols may be produced enzymatically at the intracellular level and/or from lipoprotein oxidation into the circulation (25), or by free radical-catalysed oxidation of cholesterol during digestion, both at gastric (4,26) and intestinal levels (27). Even if oxycholesterols have a faster plasma clearance than ‘normal’ cholesterol, the level of oxycholesterols in plasma can remain elevated for more than 6–8 h after a meal (18). Thus, the frequent consumption of foods rich in oxycholesterols can result in a continuous exposure during most of the day.

According to the literature, the estimates of the extent to which oxycholesterols are absorbed vary from 6 to 93% (28). This wide range of results may be due to both the dose and vehicle used to administer the oxycholesterols (28). Our estimate seems to indicate a complete absorption (105 (SD 29)% of the ingested dose) 1 h after the control meal. We hypothesise, however, that some of the oxycholesterols present in plasma derive from the oxidation of the cholesterol contained in the meal during the digestive process. Although the formation of oxycholesterols during digestion cannot be demonstrated by the present study design, several authors provide evidence that lipid oxidation can occur during digestion (26,27,29), and that the presence of antioxidants in the digestive tract can protect from this event (26,30).

A few animal studies have demonstrated that supplementation with antioxidants can prevent the increase in

| Table 1. Plasma concentration of some metabolic parameters in plasma after a double cheeseburger meal with 300 ml of water or wine (Mean values and standard deviations, n 12) |
|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                  | Control        | Wine           |                 |                |                 |                |
|                  | 0              | 1h             | 3h             | 0              | 1h             | 3h             |
|                  | Mean          | SD             | Mean           | SD             | Mean           | SD             |
| Total cholesterol (mg/dl) | 1630          | 300            | 1650           | 260            | 1610           | 300            | 1660           | 250            | 1640           | 270            | 1660           | 260            |
| TAG (mg/l)       | 820           | 160            | 820*           | 240            | 1070*          | 330            | 670            | 210            | 970            | 530            | 1230*          | 240            |
| Alcohol (% w/v)  | 0.01          | 0.01           | 0.01           | 0.01           | 0.01           | 0.01           | 0.01           | 0.01           | 0.08*          | 0.03           | 0.05*          | 0.02           |

* Mean values were significantly different from those of homologous time 0: P<0.005 (by repeated-measures ANOVA, followed by Tukey’s test).
circulating oxycholesterols induced by a high-fat diet (31), while the addition of pro-oxidant species to the diet results in a drastic increase in hepatic oxycholesterols (32). Finally, some human studies have demonstrated that long-term supplementation of antioxidants can reduce the plasma level of oxycholesterols (33). Thus, the composition of diet (its antioxidant/pro-oxidant balance) has a great influence on the circulating level of oxycholesterols.

However, in all these studies, the effects of antioxidants on the circulating level of oxycholesterols have been studied after a chronic supplementation with a high-fat diet. The present study, instead, demonstrates that wine could prevent the acute oxycholesterol 'toxicity' induced by a single high-fat meal.

It has been demonstrated that wine or wine polyphenols consumption can hinder many harmful postprandial events, such as oxidative stress and endothelial dysfunction. Red wine consumption with the meal reduces the susceptibility to oxidation of postprandial LDL (3) and prevents the postprandial increase in plasma lipid hydroperoxides and malondialdehyde (4). A standardised grape product suppresses the meal-induced impairment of vascular endothelial function (34).

In the present study, we have demonstrated for the first time that a glass of wine can prevent the postprandial increase in plasma lipid hydroperoxides and oxycholesterols after the ingestion of a high-fat, high-cholesterol meal. The peak point seems to correspond to 1 h, but our experimental design (last point 3 h after the meal) cannot indicate the length of the effect; this is a limitation of the present study.

Epidemiological studies have indicated a J-shaped relationship between wine consumption and CVD risk (35). The shape of the curve is the result of the opposite effects of wine/ alcohol on the cardiovascular system: 'positive', such as an increase in HDL-cholesterol, anti-thrombotic effects, improved endothelial function, reduced insulin resistance, etc. and 'negative', such as an increase in postprandial TAG level (that is evident also from our data, see TAG in Table 1) and induction of lipid peroxidation by ethanol.

In this view, the postprandial reduction in oxycholesterols and oxidised lipids could represent a further 'positive' effect of wine. It is well known, in fact, that oxysterols are present in atherosclerotic lesions (36–38) and atherogenic lipoprotein (39), and possess several proatherogenic activities, such as cytotoxicity on endothelial and arterial smooth muscle cells, downregulation of LDL receptors on vascular cell, proinflammatory activities (induction of cytokine release by macrophages and of the expression of adhesion molecule in endothelial cells) (40–45). Finally, several animal studies have shown that oxycholesterols promote the onset and the development of atherosclerosis (9,46,47).

The results of the present pilot study do not allow explanation of the mechanisms/reactions by which wine counteracts the postprandial increase in circulating oxidised lipids, so that we can just speculate on some possibilities, needing experimental confirmation.

Wine polyphenols and/or alcohol could minimise the postprandial increase in plasma lipid hydroperoxides and cholesterol oxidation products by (1) reducing lipid peroxidation
products or preventing their formation in the digestive tract, preventing or delaying fat absorption, (3) inducing detoxifying enzymes in the gut, and (4) enhancing the cholesterol oxidation product clearance, through the induction of enzymes involved in the cholesterol catabolism towards bile acids and/or oxysterols into the circulation after their absorption.

We studied the effect of wine as a whole, thus we cannot determine which is the wine component (alcohol or polyphenols) responsible for the observed effects and whether other forms of alcoholic beverages could have similar effects. This matter is definitely very interesting, and it should be the object of further investigation. Similarly, it should be important to study how a different ratio of wine:meal oxysterols could affect the wines capacity to cope with the increase in plasma oxysterols.

The present study provides evidence that consumption of wine with a meal could prevent and ‘counterattack’ the post-prandial increase of LDL susceptibility to oxidation. In this view, the controversial effect of lesterols, thus protecting the organism from their potential atherogenic effect. In this view, the controversial effect of lesterols, thus protecting the organism from their potential atherogenic effect. 

References
Red wine and postprandial oxycholesterols


