Wine: does the colour count?


*Department of Clinical Biochemistry, University of Toronto, Toronto, Ontario, Canada M5G 1L5
*Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8

Received 14 September 1995; accepted 20 October 1995

Abstract

The objective of this study was to determine whether phenolic constituents present in red wine and grape juice modulate plasma lipid and lipoprotein concentrations in healthy human subjects. All subjects consumed in random order 375 ml of red or white wine per day or 500 ml of two different grape juices (high and low phenols) per day for periods of 4 weeks separated by 2-week periods of abstention while continuing normal activity and food intake, and their normal lives in a community setting. The subjects were 24 healthy males aged 26–45 years screened by clinical examination and laboratory tests to exclude hypertension, diabetes mellitus, hyperlipidemia and obesity, among others. Fasting blood was collected at the beginning and end of each beverage schedule for analysis of lipids and lipoproteins. Changes in plasma lipids and lipoproteins in response to each beverage were measured to determine whether these were altered by red wine and grape juice phenolics independently of the effects of ethanol. Both grape juices had virtually no effect. Red and white wines raised plasma HDL-cholesterol and apo A-I and apo A-II concentrations as well as the apo A-I:apo B ratio to a similar extent. Red wine also raised plasma triglyceride and total cholesterol concentrations. Neither wine affected plasma apo B or apo (a) concentrations. The favourable effects of wines in modulating plasma lipid and lipoprotein concentrations are probably due to their alcohol content and cannot be reproduced by grape juices.

Keywords: Wine; Coronary heart disease; Plasma lipids; Lipoproteins; Flavonoids; Polyphenols; HDL-cholesterol

* Corresponding author
1. Introduction

The reduced incidence of mortality from coronary artery disease (CAD) among moderate consumers of beverage alcohol by comparison with abstainers has been well-documented in epidemiological studies [1–3]. Increased HDL-cholesterol accounts for at least 50% of this cardioprotective effect [4–6]. A number of lines of evidence suggest that, because of its high content of flavonoids and other polyphenolic compounds, red wine may be more beneficial than other alcoholic beverages in lowering CAD risk [7,8]. The trihydroxystilbene resveratrol has been identified as a potent modulator of lipid and lipoprotein synthesis in animal and in vitro experiments [9,10]. Since these polyphenols are also present in grape juice, this beverage merits consideration as an alcohol-free alternative to wine. The present investigation was undertaken to test if red wine phenolics promote beneficial alterations in the lipid and lipoprotein profile complementary to or independant of those induced by ethanol.

2. Subjects and methods

2.1. Beverages
White and red wines from Ontario contained trans-resveratrol <0.01 mg/l and 4 mg/l, respectively [11]. Both contained alcohol 12% by volume. Commercial grape juice was used alone, and enriched in trans-resveratrol to a concentration of 4 mg/l. These beverages were administered sequentially to all subjects as described below.

2.2. Subjects
Twenty-four healthy males aged 26–45 accustomed to consuming moderate amounts of alcohol gave informed consent to participate in this study which was approved by the Human Experimentation Committee of the University of Toronto, and were admitted after screening by extensive clinical and laboratory procedures as described [12]. Apart from the periods of wine consumption, no alcohol was permitted, and all agreed to maintain constant diet and exercise, and to refrain from any form of medication.

2.3. Experimental
The study design is summarized in Fig. 1. After 2 weeks abstinence, subjects consumed either wine (375 ml/day) or grape juice (500 ml/day) for 4 weeks. Those starting with commercial grape juice continued with the resveratrol-enriched grape juice for a further 4 weeks after which they underwent 2 weeks of abstinence before starting the wine protocol which included a 2-week period of abstinence prior to switching from one wine
Fig. 1. Chart showing beverage schedule and times of blood sampling. Arrows indicate number of weeks at end of which sample was taken to the other. The other details of the protocols were as described [12]. However, eight subjects were also monitored each week during 3 weeks abstinence after completing the final wine schedule. Blood was drawn after an overnight fast for lipid and lipoprotein assays at the beginning and end of each 4-week beverage schedule using procedures recommended by the Lipid Research Clinics [13]. The assays included plasma triglycerides and total cholesterol by enzymatic methods; HDL-cholesterol by heparin-Mg\(^{2+}\) precipitation; LDL-cholesterol by the Friedewald equation; and apolipoproteins A-I, A-II, B and (a) by immunometric procedures. They were carried out at St. Michael’s Hospital and the Toronto Lipid Core laboratory under the direction of Dr. R.L. Patten and Dr. P. Connolly, respectively.

2.4. Statistics
Mean ± S.E.M. for all assays were calculated. The statistical significance of differences between the means for basal and intervention periods in the same subjects was evaluated by the paired t-test.

3. Results
Apart from an increase in plasma apo A-II concentration after commercial grape juice (\(P < 0.01\)), neither grape juice formulation altered any of the lipids or lipoproteins measured in this study (data not shown). Table 1 summarizes the findings with the two wines for those constituents which were significantly altered by one or other or both wines. A significant increase in HDL-cholesterol and apo A-I concentrations occurred with both wines (\(P < 0.001\) for both constituents with both wines).
### Table 1
Changes in plasma lipids and lipoproteins when abstinence (2 weeks) was followed by wine consumption (4 weeks)

<table>
<thead>
<tr>
<th>Schedule</th>
<th>apo A-I (g/l)</th>
<th>HDL-C (mmol/l)</th>
<th>apo A-I : apo B</th>
<th>TG (mmol/l)</th>
<th>Total cholesterol (mmol/l)</th>
<th>apo A-II (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstinence</td>
<td>1.35 ± 0.04</td>
<td>1.16 ± 0.07</td>
<td>1.41 ± 0.10</td>
<td>1.16 ± 0.12</td>
<td>4.51 ± 0.22</td>
<td>0.266 ± 0.051</td>
</tr>
<tr>
<td>Red Wine (n = 23)</td>
<td>1.50 ± 0.04*</td>
<td>1.29 ± 0.07*</td>
<td>1.81 ± 0.11**</td>
<td>1.46 ± 0.24***</td>
<td>4.77 ± 0.22***</td>
<td>0.283 ± 0.044***</td>
</tr>
<tr>
<td>Abstinence</td>
<td>1.36 ± 0.04</td>
<td>1.15 ± 0.06</td>
<td>1.49 ± 0.12</td>
<td>1.28 ± 0.16</td>
<td>4.45 ± 0.23</td>
<td>0.272 ± 0.044</td>
</tr>
<tr>
<td>White Wine (n = 22)</td>
<td>1.46 ± 0.04*</td>
<td>1.27 ± 0.007*</td>
<td>1.61 ± 0.12***</td>
<td>1.23 ± 0.15</td>
<td>4.45 ± 0.21</td>
<td>0.291 ± 0.055**</td>
</tr>
</tbody>
</table>

Data are mean ± S.E.M. Statistical significance based on paired $t$-test compared with preceding period of abstinence:

* $P < 0.001$; ** $P < 0.005$; *** $P < 0.05$. 
Fig. 2 shows the changes in individual subjects which are numerically summarised in the legends. With both wines, the ratio apo A-I:apo B increased significantly \( (P < 0.02 \text{ for each}) \). During white wine consumption, this ratio increased in 20 and fell in three subjects; during red wine consumption it increased in 17 and fell in five (data not shown). In the eight subjects sampled weekly during 3 weeks of abstinence after the final wine schedule, HDL-cholesterol, apo A-I and the ratio apo A-I:apo B had fallen to the initial values by the end of the first week and remained relatively constant for the next two (Table 2).

Apo-AII concentrations increased significantly with red wine \( (P < 0.05) \) and white wine \( (P < 0.005) \) as shown in Fig. 2, and again returned to baseline values after one week of abstinence, remaining so for the next 2 weeks. No changes were noted in plasma LDL-cholesterol, apo B concentration, apo (a), or the ratio HDL-cholesterol:LDL-cholesterol. However, a significant increase in plasma triglycerides \( (P < 0.05) \) and total cholesterol \( (P < 0.025) \) occurred after red but not white wine consumption (Fig. 2). The former returned to initial values after 1 week of abstinence and the latter after 2 weeks (data not shown).

4. Discussion

Several authors have documented an increase in plasma HDL-cholesterol among healthy volunteers given oral alcohol over medium-term periods. Reports include increases of 40% after 70–80 g/day for 4 weeks [14], 17% in HDL3-cholesterol after 60 g/day for 3 weeks [15], 23% after 40 g/day for 6 weeks [16], and 7% after 18.4 g/day for 4 weeks [17]. One drink per day for 8 weeks did not lead to significant change in HDL-cholesterol or its HDL2 and HDL3 subfractions [18]. Reductions in

| Table 2 | Changes in HDL-cholesterol (HDL-C) and apolipoproteins during and after wine consumption in eight healthy volunteers |
|---|---|---|---|
| | Abstention (2 weeks) | Wine (4 weeks) |
| HDL-C (mmol/l) | 1.22±0.10 | 1.34±0.09 | 1.16±0.07 | 1.17±0.07 | 1.14±0.08 |
| apo A-I (mg/l) | 1.35±0.06 | 1.48±0.07 | 1.35±0.06 | 1.37±0.06 | 1.30±0.07 |
| apo A-I:apo B | 1.60±0.26 | 1.83±0.24 | 1.65±0.27 | 1.76±0.31 | 1.62±0.21 |

Data are mean±S.E.M.
Fig. 2. Panels demonstrating the changes in lipids and lipoproteins in healthy male subjects after two weeks abstinence (initial) and 4 weeks consumption of red wine. (A) HDL-cholesterol (values increased in 21 and decreased in two). (B) Apo A-I (values increased in all 23). (C) Apo A-II (values increased in 17, decreased in four and were unchanged in two). (D) Total cholesterol (values increased in 16 and decreased in seven). (E) Triglycerides (values increased in 16, decreased in six and were unchanged in one). For white wine (data not shown), the significant increases were as follows: HDL-cholesterol (increased in 17, decreased in three, unchanged in two). Apo A-I (increased in 18, decreased in three, unchanged in two), Apo A-II (increased in 16, reduced in four, unchanged in three).
plasma HDL-cholesterol have been described during periods of abstinence among alcohol abusers [19–22]. Reductions have also been reported in healthy subjects during abstention following experimental ethanol consumption [15,23]. The ethanol dose that we used was lower than in most of these investigations, yet it generated increases of 11% in HDL-cholesterol over 4 weeks which were abolished within a week of abstinence. Increased plasma concentrations of apo A-I and A-II have also been described following experimental alcohol consumption by healthy volunteers [24–27] and fell during abstention in volunteers [24] and alcoholics [19]. However, Valimaki et al. [28] found that 60 g/day but not 30 g/day ethanol over 3 weeks increased these apolipoproteins.

In our subjects, apo A-I increased by 11% and 7% with red and white wine, respectively, and fell within 1 week of abstinence. Continued steady consumption of moderate amounts of alcohol seems necessary to maintain the elevated HDL-cholesterol and apo A-I concentrations. The most striking increment with red wine was shown by plasma triglyceride concentration which rose 26% ($P < 0.05$). This was accompanied by a 6% increase in total cholesterol ($P < 0.025$). Although there was no change in the ratio HDL-cholesterol:LDL-cholesterol, on balance, red wine seems to be potentially a little less anti-atherogenic than white wine which caused similar increases in HDL cholesterol and apo A-I without increases in the potentially atherogenic triglycerides and total cholesterol.

In the only previous experimental comparison among alcoholic beverages in human subjects, Seigneur et al. reported that both red and white wine increased plasma HDL cholesterol but that neither altered apo A-I concentrations [29]. White wine increased LDL-cholesterol, whereas spirit alcohol lowered LDL-cholesterol and increased apo AI. No mechanism for these differences was proposed and no further work by these authors has been published. The present results are therefore very much at variance with those of the Bordeaux group. A paper that is frequently cited to justify the notion that red wine is more anti-atherogenic than any other alcoholic beverage described the lowest incidence of atherosclerosis in rabbits consuming this beverage along with a high-cholesterol diet [30]. The major advantage of red wine may be in its antioxidant potential which has been demonstrated both in vivo and in vitro, [31–33] and which prevents oxidation of LDL, one of the crucial mechanisms in atherogenesis [34]. Red wine flavonoids also have powerful anti-coagulation properties in vitro [35–37] and in vivo [29], and modulate eicosanoid production towards an anti-aggregatory pattern [35,37]. Despite experiments in rats showing a direct cholesterol-lowering effect of grape flavonoids and tannins, [38,39] the present results do not demonstrate any advantage of red wine over white with respect to lipid and lipoprotein metabolism,
although they do not rule out advantages in other mechanisms relevant to atherosclerosis. Our volunteers were fit and undertook regular but constant exercise throughout the study (two were trainees in physical education). This may have blunted the impact of beverage consumption upon lipoproteins as observed by Hartung et al. [23]. Grape juice, even when enriched in at least one important flavonoid, resveratrol, likewise failed to improve the lipoprotein pattern. Our findings are consistent with the epidemiological report of Klatsky and Armstrong [40] showing that those who drink only red wine have a slightly higher mortality from CAD than those who drink only white wine, although both are associated with lower risk than those who prefer spirits or beer, and lend no support to the recently published report from Denmark [41] based on self-administered questionnaires asserting that wine drinkers have lower mortality from cardiovascular and cerebrovascular disease than those drinking similar amounts of beer or spirits. If, indeed, this latter observation is correct, it is unlikely to be due to beneficial effects upon plasma lipid concentrations which have been estimated to account for at least 50% of the cardioprotective effects of moderate alcohol consumption [4–6]. Since our companion study likewise showed no advantage of red wine over white wine when platelet aggregation and eicosanoid concentrations were measured [12], the two papers are consistent in pointing to the likelihood that both wines are equally beneficial in providing protection against atherosclerosis and CHD among moderate drinkers of alcoholic beverages.

5. Key messages

- Consumption of half-a-bottle of wine daily increases plasma HDL-cholesterol and apolipoprotein A-I within a 4-week period.
- Red wine, in addition, increases plasma triglyceride and total cholesterol concentrations.
- The effects of wine cannot be reproduced by grape juices.
- Both wines modulate plasma lipids and lipoproteins towards a pattern associated with reduced risk of coronary heart disease and red wine has no advantage over white.
- The lipoprotein-modulating effects of red wine seems to be attributable to its ethanol content and is not enhanced by polyphenolic constituents.

Acknowledgements

This work was supported by grants from the Medical Research Council of Canada, The Ontario Wine Council and Welch Foods. We thank the
Liquor Control Board of Ontario for the gift of wines used in this study, and Mrs. Carole Fatah for preparing this manuscript.

References

[16] Frimpong NA, Lapp JA. Effects of moderate alcohol intake in fixed or variable


