Vive la difference! Effects of natural and conventional wines on blood alcohol concentrations: a randomized, triple-blind controlled study.

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Abstract: Different alcoholic beverages can have different effects on blood alcohol concentrations (BAC) and neurotoxicity even if equalized for alcohol content by volume. Anecdotal evidence suggested that natural wine is metabolized differently from conventional wines. This triple-blind study compared the BAC of 55 healthy male subjects after consuming the equivalent of 2 units of alcohol of a natural or conventional wine over 3 mins in two separate sessions one week apart. BAC was measured using a professional breathalyzer every 20 mins after consumption for 2 hrs. The BAC curves in response to the two wines diverged significantly at twenty minutes, at forty minutes and also at their maximum concentrations (peaks), with the natural wine inducing a lower BAC than the conventional wine (T20 0.40 vs. 0.46 [p<0.0002], T40 0.49 vs. 0.53 [p<0.0015], peak 0.52 vs. 0.56 [p<0.0002]). These differences are likely related to the development of different amino acids and antioxidants in the two wines during their production. This in turn may affect the kinetics of alcohol absorption and metabolism. Other contributing factors may also include pesticide residues, differences in dry extract content and the use of indigenous or selected yeasts. Further studies are needed to fully understand why natural wines are metabolized differently from conventional wines.

Keywords: alcohol; natural wine; blood alcohol content; breathalyzer; pesticides

1. Introduction

In recent decades, wine consumption has been the subject of intense debate within the scientific community. On the one hand, wine has been linked to reduced risk for several chronic illnesses, such as cardiovascular diseases, osteoporosis and...
diabetes [1]. On the other hand, international guidelines for cancer prevention emphasize the direct correlation between alcohol intake and cancer risk [2, 3]. The positive health benefits provided by wine come primarily from compounds called polyphenols, which are natural antioxidants that help fight inflammation and improve plasma lipid profiles [4]. When consumed regularly and moderately, ethanol, the main alcohol component in wine, confers cardioprotective effects by acting directly on cardiomyocytes, blood circulation and platelet aggregation [5]. However, ethanol and its metabolite acetaldehyde are also responsible for adverse neurological, hepatic and oncological consequences secondary to alcohol consumption [6, 7]. Because of its potentially beneficial and harmful effects, many scientific organizations recommend that alcohol consumption be limited to lower-alcohol beverages, such as wine [8], and that such beverages be consumed moderately and responsibly, if at all [9, 10].

In Italy, the Research Centre for Food and Nutrition (CREA-AN) of the Council for Agricultural Research and Economics has adopted guidelines issued by the National Institute for Research on Food and Nutrition (INRAN), which define moderate alcohol consumption as an average daily allowance of no more than 2-3 units of alcohol for men, and 1-2 units for women. The standard value of a unit of alcohol in Italy is 12 g of ethanol [11].

As reported by the Italian National Institute for Research on Food and Nutrition, there is a well-known linear correlation between blood alcohol concentration BAC and the deleterious effects of alcohol, particularly those involving the central nervous system [11]. The short-term neurotoxic effects of elevated BAC include a state of euphoria or inebriation, slowed reflex and reaction times, diminished peripheral vision, and cognitive impairment [12].

The relationship between the amount of alcohol consumed and BAC is influenced by numerous factors, including the individual’s sex, age, body weight, liver volume and function, drinking habits, use of medications, medical conditions, and fasting or non-fasting state [13].

It has also been established that, when equalized for alcohol content by volume, different beverages are absorbed at different rates and lead to different maximum concentration (peaks) in BAC [14, 15]. This study set out to determine whether the absorption of ethanol from two wines produced from the same grape (with similar alcohol and sugar content) might be affected by differences in the farming and winemaking techniques used in their production. The approach is therefore to compare the evolution of BAC of healthy male subjects after their consumption of 2 units (24 g of alcohol) of a natural wine (i.e., cultivated without pesticides and agrochemicals, fermented with wild yeasts, unfiltered and with no fining; see Materials and Methods) and after their consumption of the same amount of a conventional wine, a week apart and under the same experimental conditions.

2. Materials and Methods

2.1. Selection of the natural and conventional wines
In the absence of clear national or international legislation on the definition of natural wine, it was decided for the purpose of this study to compare the effects of consuming two near-identical wines differing only in the farming management and vinification protocols adopted in their production. Over three hundred wines were purchased for testing by an independent laboratory specialized in alimentary analysis to identify those suitable for comparison. Our intention was to find a pair of wines, one natural and one conventional, with near-perfect (or the best-possible) correspondence in grape variety, proximity of the area of production, age of harvesting, alcoholic strength by volume, and low sugar content (<1.5 g/L). Two wines satisfied the inclusion criteria. Both were whites made from Cortese grapes grown in vineyards located within ten kilometers of each other in Piedmont, Italy. They were of the same vintage and aged in bottles for twelve months. Table 1 shown the main characteristics of the two wines selected for the trial.

Table 1. Characteristics of the natural and conventional wines tested.

<table>
<thead>
<tr>
<th></th>
<th>Natural wine</th>
<th>Conventional wine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual alcoholic strength by volume (vol%)</td>
<td>13.19</td>
<td>13.03</td>
</tr>
<tr>
<td>Volatile acidity (mEq/L)</td>
<td>20</td>
<td>4.5</td>
</tr>
<tr>
<td>Total sugar content (g/L)</td>
<td>&lt; 1.5</td>
<td>&lt; 1.5</td>
</tr>
<tr>
<td>Total dry extract (g/L)</td>
<td>24.93</td>
<td>18.23</td>
</tr>
<tr>
<td>Total sulfur dioxide (g/L)</td>
<td>0.025</td>
<td>115</td>
</tr>
<tr>
<td>Pesticides</td>
<td>n.d.</td>
<td>present</td>
</tr>
</tbody>
</table>

As can be seen from the table, the two wines had the same percent of alcohol by volume and a low sugar content (<1.5 g/L), and both were made from the same variety of grape grown in the same geographic location. The grapes used for making the natural wine, however, were cultivated without pesticides or agrochemicals other than those approved for organic farming by EC Reg. 834/2007 [16]. The wine was fermented without the use of selected yeasts or fining processes. The wine was also left unfiltered and no sulfites were added. Instead, the grapes for making the conventional wine were grown using regulated synthetic pesticides and agrochemicals, and fermented with selected yeasts, and the entire winemaking process was based on conventional methods permitted by Italian law, including filtration and the addition of sulfur dioxide. The wines were subjected to additional tests for pesticides, confirming traces of Iprovalicarb (45 µg/kg) and Fenhexamid (120 µg/kg) in the conventional wine.

2.2. Study design

The study was a randomized, triple-blind, controlled trial. Each phase of the trial was conducted in triple-blind, meaning that at no time were any of the three research teams aware of the identity of the wines being administered to the subjects or being analyzed. The first team designed the study, selected the volunteers and set up the
samples for testing. The second administered the doses of wine to the subjects and recorded the resulting data, and the third conducted the final data analysis. Among the 167 eligible subjects, 55 subjects were drawn by a random number generator. In addition, at the start of the test, the matrices containing the matching between the subjects and the bottles were generated by a random number generator, as was the assignment one of the two types of wine to each subject in the first or second week.

2.3. Subjects

All participants in the study were university student volunteers, screened using a questionnaire to collect data on their height, weight, body mass index (BMI), dietary habits, and use of prescription medicines. Ethical approval was provided by the Polytechnic University of Turin (1037/2018, 01-30-2018) in compliance with the Helsinki Declaration. Recruitment was limited to male subjects, as the study required consumption of 2 units of alcohol, which exceeds the maximum recommended daily amount for women (INRAN, 2003). At the end of the study, an information and awareness campaign was carried out to promote alcohol awareness and responsible use among the entire student body at the Polytechnic University of Turin. Inclusion criteria: Males aged 18 to 30 with a BMI between 18.5 kg/m² and 25 kg/m² who could understand the purpose of the study and thus provide written informed consent. Exclusion criteria: Use of prescription medicine for chronic conditions; any pathology that might interfere with alcohol metabolism; habitual consumption of more than 4 units of alcohol per day.

2.4. Administering the two wines

The test took place over two sessions held one week apart. At the first session, each subject was given 3 min to drink a single, unlabeled and randomly selected 248 ml dose of either the natural wine or the conventional wine (the equivalent of 2 units, or 24 g of alcohol). At the second session, seven days later, the subjects had to repeat the experiment, this time being administered the other type of wine to drink.

Table 1 summarizes the characteristics of the two wines tested. While similar in alcohol strength by volume and sugar content, they showed significant differences in volatile acidity, total dry extract, and sulfur dioxide concentrations, which can be attributed to the different farming and vinification processes used in their production.

After providing breath samples at the beginning of each session to verify a zero BAC, the subjects underwent a series of breathalyzer tests to measure their blood alcohol levels at 20-min intervals for a total of 2 h after ingesting the sample (time intervals T0, T20, T40, T60 T80, T100, T120). A professional AlcoTrue M breathalyzer (bluepoint MEDICAL, Selmsdorf, Germany) was used in order to infer the BAC.

The subjects were required to abstain from drinking alcohol for 7 days, from smoking for 8 h and from eating for at least 4 h prior to both sessions for minimizing the possible interference of assuming alcohol, smoke or food during the previous week and the previous hours. They were deliberately provided with no information
about the wines being tested, and the wines were served at 21°C and the same
temperature was maintained in the testing room throughout the test to prevent any
temperature-dependent interference on the BAC [17]. The room had artificial
lighting and the two tastings took place at the same time of day to minimize any
possible interference. The wines were distributed in plain black wine tasting glasses.
The labels on the bottles were masked, and the wines were identified strictly by a 4-
digit code number. The first two digits indicated the day on which the test was
performed, while the second two indicated the bottle index randomly assigned to
each bottle.

2.5. Pharmacokinetic analysis

A professional breathalyzer was used to estimate the following pharmacokinetic
parameters: BACs expressed in g/L at time intervals T0, T20, T40, T60, T80, T100,
T120; maximum BAC (peak)-versus time, the ethanol’s area under curve (AUC)
calculated using the trapezoidal rule.

2.6. Statistical analysis

The sample size was calculated on the basis of the main expected outcome,
defined as the difference between BAC after drinking a fixed dose of natural wine
and after drinking the same dose of conventional wine. Using data in the literature
on subjects similar to those participating in our study, it was calculated that for an
effect size of 0.67 and two tailed alpha-error of 0.05, 50 subjects could be needed to
obtain 90% of power. As a precautionary measure, the sample size was set at 55
subjects. Continuous variables were expressed as medians and interquartile ranges
(IQR), and categorical variables as percentages and absolute frequencies. The
Student’s t-test for paired samples was used to detect differences in BAC at each of
the time intervals (T0, T20, T40, T60 T80, T100, T120) and to detect differences in
AUC, too. The resulting data was graphically represented using box-and-whisker
plots. The level of significance was set at p ≤ 0.05. All statistical analysis was
performed with the MedCalc Statistical Software version 18.9 (Ostend, Belgium).

3. Results

The 55 male subjects recruited for the study were of median age 23 (p25: 21 years,
p75: 24 years), median weight 69 kg (p25: 65 kg, p75: 78 kg), median height 178 cm
(p25: 174 cm, p75: 183 cm), and median BMI 22 kg/m² (p25: 20.8 kg/m² cm, p75: 22.9
kg/m²). Breathalyzer measurements obtained at regular twenty-minute intervals
were used to plot concentration-time curves of each subject’s BAC response to the
natural wine and to the conventional wine. These can be seen in Appendix A,
Figures A1 to A5. Superimposition of the pairs of curves reveals that each subject
had its own distinct pattern of alcohol pharmacokinetics to both wines, as is evident,
for example, for subjects 21, 25, 43 and 44, randomly extracted from the sample
(Figure 1).
Figure 1. Examples of blood alcohol concentration (BAC) levels measured every 20 mins after the ingestion of the conventional wine (CW) and of the natural wine (NW) (g/L), subjects #21, #25, #43 and #44.

By summing all of the subjects’ BAC values at the different time intervals and dividing by the number of subjects, the average BAC curves were calculated. Figure 2 shows the differences in the average BAC levels registered after the ingestion of the natural vs. the conventional wine.
Figure 2. Comparison of the average blood alcohol concentration (BAC) levels measured every 20 mins after the ingestion of the conventional wine (CW) and of the natural wine (NW) (g/L).

It can be seen that the rate of increase in BAC in response to the two wines diverges significantly at the T20 mark, with natural wine inducing lower levels than conventional wine: 0.40 vs. 0.46 (p<0.0002) (Figure 3); this discrepancy persists at significant levels up to T40: 0.49 vs. 0.53 (p<0.0015) (Figure 4).
Figure 3. Box and whiskers diagram of the blood alcohol concentration (BAC) levels at T20 after the ingestion of the conventional wine (CW) and of the natural wine (NW) (g/L) (T20 p<0.0002).
Figure 4. Box and whiskers diagram of the blood alcohol concentration (BAC) levels at T20 after the ingestion of the conventional wine (CW) and of the natural wine (NW) (g/L) ($p<0.0015$).

The BAC peaks occur between T40 and T60 for both wines. The difference in values of the maximum BAC levels after the ingestion of the natural wine (NW) is significantly lower than after the ingestion of conventional wine (CW) 0.52 vs. 0.56 ($p<0.0002$) (Figure 5). The curves continue to approach each other until the T80 mark and then intersect. After this point the conventional wine is associated with a slightly lower BAC and the curves gradually converge and largely overlap toward the end.
Figure 5. Box and whiskers diagram of the maximum blood alcohol concentration (peak) levels after the ingestion of the conventional wine (CW) and of the natural wine (NW) (g/L). (p<0.0002).

The AUC was calculated from T0 to T120 using the trapezoidal method. This parameter proved not to be significant (p=0.13). This means that although the increasing BAC in response to the two wines differs at specific points along the curve, the overall variation does not reach significance when the curve is considered as a whole (Figure 6).
It is interesting to note that, with the exception of the peak, the median BAC measured in response to the natural wine is consistently below 0.5 g/L, the maximum legal drink driving limit in many countries. In contrast, the BAC in response to conventional wine not only exceeds the legal driving limit at its maximum peak, but also approaches it at T20 and exceeds it at T40.

4. Discussion

Our findings indicate that the peak BAC reached after drinking a natural wine is significantly lower than after drinking the same amount of a conventional wine with a similar total alcohol strength by volume. The alcohol in natural wine is absorbed more slowly than that in conventional wine, as can be seen by the discrepancy between the BAC measurements at T20 and at T40 (Fig. 2). Ethanol is absorbed into the blood stream mainly through the jejunum via passive diffusion, and down the concentration gradient between the small intestine and the capillaries [13]. Numerous factors can influence the absorption rate of alcohol: the type of beverage and manner of ingestion (total alcohol content, the concentration of alcohol, whether or not it is consumed as a single dose or as multiple smaller doses), as well as the intrinsic characteristics of the subject (mucosal integrity of the intestine, efficient blood flow, the presence or absence of food in the stomach, and alcohol dehydrogenase activity in the gastric mucosa) [13]. This study was designed to rule out possible causes for differences related to the mode of consumption or the
intrinsic characteristics of the subjects. The causes can thus be attributed to
differences in the non-alcoholic component of the two wines.

Chemico-physical analysis of the samples revealed substantial differences in the
total dry extract of the two wines. This is a direct consequence of differences in
farming and winemaking practices, with the absence of filtration processes in
natural wine likely to be a key factor. The total dry extract of a wine contains all of
its non-volatile substances, such as sugars, polyphenols, fibers and minerals. The
total dry extract contained in the dose of natural wine was 1.67 g higher than in the
conventional wine used for testing. This may affect gastric emptying time and,
consequently, the absorption rate of ethanol [18].

The total sulfur dioxide content in the two wines also differed, with conventional
wine containing the larger share. Sulfur dioxide has antioxidant and antiseptic
properties that inhibit the growth of certain strains of yeast and bacteria during the
various phases of winemaking [19]. Although the in vivo metabolic effects of sulfur
dioxide have been widely studied [20], there have been no reports on its
involvement in the absorption or metabolism of alcohol.

Another important distinction between natural and conventional wines lies in
the vinification process. Natural wine is the product of spontaneous fermentation by
indigenous yeasts naturally found on the grapes, while conventional wines are
produced using mixtures of laboratory-selected microorganisms. The presence of
various strains of bacteria and yeasts during fermentation results in the
development of different metabolites [21, 22]. At present, the results of chemico-
physical analysis of the samples used in this study are unable to provide precise
information about these differences. Additional data may emerge thanks to the use
of new technologies. In recent years, for example, high-field $^1$H nuclear magnetic
resonance ($^1$H-NMR) spectroscopy has allowed detailed investigation of wine
metabonomics [23] and has demonstrated that the vinification protocol is one of the
chief factors determining the amino acid, alcohol and polyphenol make-up of two
wines from the same geographic location [24]. Another study established that
different production chains determine variations in the amount and type of
antioxidants found in organic and biodynamic wines [25]. Comparable data on
wines produced using the natural winemaking process are not yet available.

Besides producing wines with different amino acid and polyphenolic profiles,
differences in the natural and conventional fermentation pathways may also
generate other molecules that interact with absorption or with specific isoforms of
alcohol dehydrogenase (ADH), the enzyme involved in breaking down alcohol. This
would in turn lead to differences in the rates of metabolism of natural wine and
conventional wine.

The polyphenolic content of wine has been found to alter the intestinal
microbiota by stimulating growth of bifidobacteria and lactobacilli and decreasing
the numbers of clostridia and enterobacteria [26, 27]. Prolonged alcohol abuse, on
the other hand, can produce a state of intestinal dysbiosis with overgrowth of
proteobacteria [28]. It is unlikely, however, that these differences affect the
absorption and metabolism of alcohol in the short term.
It seems reasonable to expect the polyphenolic profile of two differently produced wines to have dissimilar effects on the individual’s microbiota. In any case, the wines used in this study were white, meaning they were not as rich in antioxidants as reds and rosés, so any variability due to the total content of antioxidants (particularly of resveratrol) was minimized [29].

A further possibility is that pesticide residues (Tab. 1) might interfere with the absorption, metabolism and pharmacokinetics of alcohol in conventional wines, where contaminant analysis has revealed traces of the fungicides iprovalicarb and fenhexamid. Both are present within legal limits [30, 31] and there have been no reports of acute intoxication or known effects on liver metabolism caused by their presence in wine [32, 33]. However, understanding the toxicity of pesticides and their interaction with metabolic processes in vivo is extremely complex, given the vast number of simultaneously interacting molecules [34]. Therefore, it cannot be ruled out that synergistic interactions among the different contaminants might influence the absorption or metabolism of ethanol.

The different kinetics observed for natural wine and conventional wine may have important clinical implications. Acute alcohol intoxication is one of the leading causes of emergency room visits [35], and approximately 5% of deaths from acute poisoning are attributable to alcohol [36]. Systemic toxic effects are proportional to BAC, and levels above 0.5 g/L are enough to impede normal daily activities. Concentrations above 4 g/L cause hypoventilation, which, if untreated, can lead to coma and death [37]. Our findings show that the peak BAC in response to natural wine is lower than that to conventional wine, meaning that natural wine is less likely to lead to alcohol intoxication.

Despite numerous mass media campaigns to promote responsible drinking, alarming epidemiological evidence shows that they are largely ineffective in causing a reduction in alcohol consumption [38]. According to 2017 data from the Italian National Institute of Statistics (ISTAT), regular daily consumption of alcohol with meals is slightly on the decline, whereas occasional or irregular drinking outside of meals and binge drinking, particularly among youths below the age of 25, has increased dramatically [39].

It is estimated that 35% of road fatalities are linked to alcohol. Because of the dangers of alcohol-induced cognitive impairment, most European countries have passed laws making it an offense to drive with a BAC in excess of 0.5 g/L. The fact that in our study, among those subjects who drank 2 units of natural wine, only 56% exceeded the legal blood alcohol limit of 0.5 g/L, as opposed to 67% of those who drank the same amount of conventional wine, suggest further investigations.

A preliminary study by Bassani et al. (Marco Bassani, personal communication, September 11th, 2018) compares the behavior of subjects who consumed natural wine or conventional wine prior to completing a simulated driving task. Subjects who drank conventional wine before the simulation tended to drive more aggressively than those who consumed an equal amount of natural wine. In particular, the natural wine drinkers drove consistently slower and committed fewer traffic violations than those who drank conventional wine.
5. Conclusions

To our knowledge, this is the first scientific study to compare the pharmacokinetics of alcohol of a conventional wine with that of a nearly identical natural wine. It analyzed the effects on BAC of drinking a natural wine or of an equal amount of conventional wine, both from the same terroir and containing the same percent of alcohol by volume and total sugars. The BAC level 20 mins and 40 mins after drinking the natural wine was lower than that after drinking the conventional wine, and the peak blood alcohol response to drinking natural wine was also lower than the peak response to drinking conventional wine. This supports the hypothesis that natural and conventional wines are metabolized differently.

The key strengths of this study are its randomized, triple-blind, controlled design and its careful selection of the wines, both of which came from the same variety of grape and were virtually identical in many of their physical and chemical characteristics. Recruitment of a homogeneous group of subjects reduced the variability in individual kinetics and alcohol metabolism. A limitation of the study is the brief period (2 h) allotted to measuring the subjects’ BAC, and future trials may wish to extend the time used for testing.

More work is needed to fully understand the relationship between natural wine and BAC. Currently, little data is available on other types of wine (red and rosé) and other segments of the population (women and the elderly). However, studies using higher doses of wine would pose ethical challenges related to exposing subjects to more alcohol than is considered safe. Additional studies using new technologies such as $^1$H-NMR will make it easier to pinpoint differences in the chemical composition of natural and conventional wines.

In the absence of specific laws and more precise laboratory data, the differences between natural wine and conventional wine must be imputed to differences in agricultural methods, winemaking processes, and preservation techniques, and their description is mainly relegated to expression of the consumers’ sensorial experience of the final product. The present study has been able to confirm that there are indeed objective differences in the absorption of natural wine and conventional wine.

Further research would be useful with a view to developing a universal legislative framework for the regulation of natural wines and for shedding light on differences in the public health implications of natural wine and conventional wine. Because it leads to a lower peak BAC than conventional wine, natural wine may be linked to a lower risk for alcohol intoxication. If future studies confirm this hypothesis, every effort should be made to include this information in public awareness and educational campaigns about responsible drinking. Given the growing international interest in natural wines [40, 41] as well as consumers’ increasing demand for “natural” alcoholic beverages with a low environmental impact, further research should be undertaken to better understand the potential health benefits provided by natural wines.
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Appendix A. Concentration-time curves of each subject’s BAC response to the natural wine and to the conventional wine (Figures A1 to A5).

Figure A1. Concentration-time curves of BAC responses to the natural wine and to the conventional wine for Subjects #1-#12.
Figure A2. Concentration-time curves of BAC responses to the natural wine and to the conventional wine for Subjects #13-#24.

Figure A3. Concentration-time curves of BAC responses to the natural wine and to the conventional wine for Subjects #25-#36.
Figure A4. Concentration-time curves of BAC responses to the natural wine and to the conventional wine for Subjects #37-#48.

Figure A5. Concentration-time curves of BAC responses to the natural wine and to the conventional wine for Subjects #49-#55.

References


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