Inverse correlation between alcohol consumption and lymphocyte levels of 8-hydroxydeoxyguanosine in humans

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In a cross-sectional study of 115 premenopausal non-smoking women, we examined the relationship between lymphocyte levels of 8-hydroxy-2'-deoxyguanosine (8-oxodGuo) and habitual alcohol consumption. The study was conducted in four different regions of Europe, including Potsdam (Germany), Turin (Italy), Malmö (Sweden) and Granada (Spain). Mean 8-oxodGuo levels differed significantly across study centres (P = 0.001), with the highest levels in Granada [2.17 8-oxodGuo × 10⁻⁶ 2'-deoxyguanosine (95% confidence interval 1.27–4.40)] and lowest levels in Turin [1.19 (0.36–4.29)]. Mean levels of total alcohol intake and of types of alcoholic beverages consumed (wine, fortified wines, beer and cider) also differed across the study centres (P < 0.05), with the highest total alcohol consumption in Turin, and the lowest intake in Granada. When combining all the data, but adjusting for study centre, individual 8-oxodGuo level correlated inversely with alcohol intake. This inverse association remained unaltered after further adjustment for Quetelet Index, fruit and vegetable consumption, and plasma carotenoid levels. Furthermore, the inverse association was also observed for each of the study centres separately, and for different beverage types, with the exception of Granada, where the majority of women were non-drinkers and where alcohol intakes were also very low for the consumers. Finally, on a group level, mean levels of 8-oxodGuo and alcohol intake were also inversely associated between the four study centres. The finding of a relationship between alcohol consumption and 8-oxodGuo in lymphocytes was unexpected and not based on a prior hypothesis. This finding consequently requires confirmation from a randomized intervention study.

Oxidative damage to macromolecules, including DNA, proteins and lipids, has been implicated in the etiology of aging and various age-related diseases, including cardiovascular diseases and cancer (1). Several studies have shown that these forms of oxidative damage can be modulated by environmental and dietary factors. For example, cigarette smoking has been found to have a pro-oxidant effect, and was positively associated with levels of 8-hydroxy-2'-deoxyguanosine (8-oxodGuo) in lymphocyte DNA (2), and with urinary levels of 8-oxodGuo (3) and 5-hydroxymethyluracil (4). Among dietary factors, a rise in dietary fat intake was found to increase leukocyte levels of 5-hydroxymethyl-2'-deoxyuridine (5), whereas supplementation with antioxidants (6,7), carrot juice (8) or brussel sprouts (9) decreased levels of oxidative damage measured in lymphocytes or urine. Nevertheless, there have also been paradoxical findings. For example, we recently found that lymphocyte levels of 8-oxodGuo were not lower, but higher in a group of women consuming a typical Mediterranean diet, rich in natural antioxidants such as vitamin C, carotenoids and tocochromers compared with women with a Northern European dietary intake pattern (10). To investigate further the relationships between habitual diet and oxidative DNA damage, we extended this study to two additional research centres, Potsdam (Germany) and Turin (Italy). In this paper we report some unexpected findings, relating habitual moderate alcohol consumption to reduced lymphocyte levels of 8-oxodGuo.

Premenopausal women, aged 45–50, previously recruited for the European Prospective Investigation into Cancer and Nutrition (EPIC; a multi-centre prospective cohort study on diet and cancer), were selected from four of the EPIC study centres, in Germany (Potsdam), Italy (Turin), Sweden (Malmö) and Spain (Granada). All the women selected were non-smokers, and none of them had used any vitamin supplements, or had any form of chronic inflammatory disease, hypertension or diabetes. Information on habitual diet was obtained by previously validated food frequency questionnaires (11). The amount (ml) of alcohol consumed with different alcoholic beverages was computed assuming 11 vol% alcohol in wine, 16 vol% in fortified wines, 4 vol% in beer and cider and 40 vol% in spirits and brandy. A fasting blood sample, containing heparin as anticoagulant, was collected for laboratory analyses. Immediately after the blood draw, plasma and lymphocytes were prepared by centrifugation on Lymphoprep tubes (Nycomed Pharma AS, Oslo, Norway) and stored at –80°C.

Lymphocyte levels of 8-oxodGuo were measured in a single laboratory at IARC by HPLC with electrochemical detection, as previously described (10). The analyses were performed in batches of 12 DNA samples per day; each batch included in random order an equal number of subjects either from Malmo and Granada, or from Potsdam and Turin. The mean storage times before analyses were, respectively, 30.34 ± 2.49 and 29.70 ± 2.38 months for Malmö and Granada, and 18.09 ± 1.69 and 11.31 ± 1.75 months for Potsdam and Turin, respectively. In order to avoid artefactual oxidation of guanine, especially during the DNA extraction steps (12), 0.1 mM deferoxamine was added to all buffers. In addition, samples were always processed on ice and all centrifugation

Abbreviations: dG, 2'-deoxyguanosine; 8-oxodGuo, 8-hydroxy-2'-deoxyguanosine; EPIC, European Prospective Investigation into Cancer and Nutrition; GLM, generalized linear regression models.
steps were performed at 4°C. In some samples 8-oxodGuo was not detectable, either because the amount of extracted DNA was not sufficient for the chemical analysis or because the chromatographic peak of 8-oxodGuo presented some interference. These subjects were excluded from statistical analyses. These exclusions, however, did not change significantly the average subject characteristics of age, height, body weight or mean levels of alcohol consumption. Carotenoids and α-tocopherol were measured in plasma using HPLC with a diode array detector (13).

The data were analysed by generalized linear regression models (GLM), with 8-oxodGuo levels as a dependent variable, and ‘centre’, storage time and total alcohol consumption as predictor variables. Additional variables added to the models for further adjustments included Quetelet Index (weight/height²), total fruit and vegetable consumption, and plasma total carotenoids (the sum of α-carotene, β-carotene, β'-cryptoxanthin, canthaxanthin, lutein, lycopene, zeaxanthin and retinol), and plasma α- and γ-tocopherols. All analyses were performed on 8-oxodGuo values that had been log-transformed to normalize their population frequency distributions.

Basic characteristics of study participants and means and ranges of 8-oxodGuo are in Table I. Women had the same average age in all four centres, but had a lower Quetelet Index in Turin and Potsdam than in Granada (P < 0.0004), and were taller in Potsdam, Turin and Malmö than in Granada (P < 0.0001). Levels of 8-oxodGuo ranged from 0.36 to 6.23 8-oxodGuo×10⁻⁶ 2'-deoxyguanosine (dG), and thus showed high inter-individual variation, consistent with previously reported data (2,14,15). On an absolute scale, these levels were relatively low compared with those measured in other laboratories by similar techniques (14–17). These relatively low absolute levels suggest that there was not considerable artefactual formation of 8-oxodGuo during sample processing and analysis. Geometric means in the four study centres were 1.29 8-oxodGuo×10⁻⁶ dG (95% confidence interval 0.96–1.72) for Potsdam, 1.19 (0.96–1.48) for Turin, 1.31 (1.01–1.70) for Malmö and 2.17 (1.92–2.46) for Granada. Analysis of variance by GLM models (see also below) indicated that the mean levels differed significantly between the four centres (P = 0.001), with the strongest contrast between Granada and the other three centres. Average sample storage times differed between the centres, but within centres showed no significant correlation with 8-oxodGuo levels.

Mean alcohol intake calculated for all subjects (either drinkers or non-drinkers), was highest in Turin, where alcohol intake was mainly derived from wine, whereas intake was particularly low in Granada (Table II). The low average alcohol consumption in Granada was mainly due to a relatively large proportion of non-drinkers: only 12 out of 28 women in Granada reported drinking alcoholic beverages; nine reported drinking wine and only four reported drinking beer. On the contrary, all subjects in Potsdam were drinkers and only five out of 36 and seven out of 24 were non-drinkers in Turin and Malmö, respectively. In Potsdam and Malmö levels of total alcohol consumption were quite similar, and in these two centres beer consumption contributed to a higher percentage of total alcohol intake than in Turin. Differences between the four centres in the consumption levels of total alcohol were highly significant (P = 0.001), while there were also clear differences in the consumption levels of specific beverages.

The relationship between alcohol intake and 8-oxodGuo level was analysed both at the level of individuals, with adjustment for study centre, and at the level of study centres by comparing average alcohol intakes and mean 8-oxodGuo levels for each of the four study populations. At the individual level, a negative relationship between 8-oxodGuo and total alcohol intake was observed in three centres (Malmö, Potsdam and Turin) where most women reported regular alcohol consumption (Figure 1). In addition, when comparing mean alcohol intakes and mean 8-oxodGuo values between the four study centres an inverse association on a group level appeared (Figure 2).

Multivariate analysis by GLM, all study subjects combined (n = 115), showed a significant effect of the variable ‘study centre’ (P = 0.001), which explained about 13% of variation in 8-oxodGuo levels (model R-square of 0.134) (Table III). Entering total alcohol intake into the model diminished the effect of ‘study centre’ (partial R-square of 0.063), but showed a significant effect of alcohol intake (P = 0.02), with a partial R-square of 0.043 adjusted for the centre effect. For alcohol intake (a continuous variable), the square root of this partial R-square indicates the average correlation within study centres of 8-oxodGuo with alcohol intake (−0.21). The inverse association between alcohol intake and 8-oxodGuo levels remained unaffected after further adjustments for sample storage time, Quetelet Index, total fruit and vegetable consumption, and plasma levels of total carotenoids and tocopherols. It is worth noting that in the latter models sample storage times was unrelated to 8-oxodGuo levels; however, total plasma carotenoids and tocopherols were positively associated with 8-oxodGuo levels, confirming our earlier observations for Malmö and Granada only (10). Considering different beverage types, 8-oxodGuo levels were inversely related to levels of consumption of all types of beverage combined, the negative correction being more important, although not always significant for beer and spirits in Potsdam, wine and fortified wines in Turin, wine in Malmö and beer in Granada (Table

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**Table I. Subject characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Potsdam (n = 27)</th>
<th>Turin (n = 36)</th>
<th>Malmö (n = 24)</th>
<th>Granada (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47.4 ± 1.6⁸</td>
<td>47.7 ± 1.8⁸</td>
<td>47.8 ± 1.6⁸</td>
<td>47.9 ± 1.6⁸</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.0 ± 4.8⁸</td>
<td>161.2 ± 6.7⁴</td>
<td>164.7 ± 6.2⁸</td>
<td>157.8 ± 4.6⁸</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.1 ± 11.7⁸</td>
<td>61.7 ± 8.2⁴</td>
<td>73.0 ± 15.1⁸</td>
<td>68.8 ± 9.3⁸</td>
</tr>
<tr>
<td>Quetelet Index</td>
<td>25.3 ± 4.3⁸</td>
<td>23.7 ± 2.9⁶</td>
<td>26.8 ± 4.8⁸</td>
<td>27.6 ± 3.1⁶</td>
</tr>
<tr>
<td>8-OxodGuo (×10⁻⁶ dG)</td>
<td>1.29 (0.37-6.23)</td>
<td>1.19 (0.36-4.29)</td>
<td>1.31 (0.41-4.30)</td>
<td>2.17 (1.27-4.40)</td>
</tr>
</tbody>
</table>

⁸Means ± SD.
⁹Geometric mean; numbers in brackets are the minimum and maximum values.
¹⁰Mean values of centres with different alphabetical letters are significantly different (P < 0.05).
Table II. Daily consumption (ml) of alcohol from different alcoholic beverages in Potsdam, Turin, Malmö and Granada (mean ± SD)

<table>
<thead>
<tr>
<th>Alcoholic beverage</th>
<th>Potsdam (n = 27)</th>
<th>Turin (n = 36)</th>
<th>Malmö (n = 24)</th>
<th>Granada (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>9.9 ± 9.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.1 ± 15.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.7 ± 11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wine</td>
<td>6.7 ± 9.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>11.4 ± 15.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5 ± 6.3&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.6 ± 1.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fortified wines</td>
<td>0.05 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.2 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Beer, cider</td>
<td>3.0 ± 4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.7 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.0 ± 4.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Spirits, brandy</td>
<td>0.2 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.7 ± 1.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.2 ± 3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>–</td>
</tr>
</tbody>
</table>

<sup>ab</sup>Mean values of centres with different alphabetical letters are significantly different (P < 0.05).

Fig. 1. Relationships between an individual’s alcohol intake and 8-oxodGao levels in lymphocyte DNA, within each of the four study centres.

IV). This suggests that the inverse association of alcohol intake with 8-oxodGao levels was independent of beverage type.

In summary, we observed a negative relationship between level of alcohol intake and oxidative DNA damage, as measured by 8-oxodGao levels in lymphocytes. This finding was unexpected, and was not based on a prior hypothesis. The inverse relationship was most evident in three of the four centres (Potsdam, Turin and Malmö) where the proportions of alcohol drinkers were high. In addition, mean alcohol intakes were correlated inversely with mean 8-oxodGao levels across the four study populations. Interestingly, this inverse association between alcohol intake and oxidative DNA damage appeared to be independent of beverage type.

The mechanisms that may underlie our findings are not clear. On the one hand, ethanol is known as a potent hydroxyl radical scavenger in vitro (18). On the other hand, several experimental studies in vivo have shown pro-oxidant effects of pure ethanol. In rats, ethanol feeding increased the production of reactive oxygen species, lipid peroxidation and DNA fragmentation in the gastrointestinal tract (19), increased 8-oxodGao levels in hepatic mitochondrial DNA (20,21) and induced DNA strand breaks in brain tissue (22). Likewise, in mice, intragastric doses of ethanol increased lipid peroxidation and degradation of hepatic mitochondrial DNA (23). In contrast, a moderate consumption of beer did not affect plasma antioxidant status in rats, but increased their resistance to lipid peroxidation (24), whereas another study showed no difference in the oxidative status of rats supplemented with wine (25).

Studies in humans on the effect of alcohol consumption on oxidative damage also produced contradictory results. A positive correlation between alcohol intake and levels of 8-oxodGao in lymphocytes was found in subjects deficient in aldehyde dehydrogenase-2 (14), an enzyme that metabolizes the alcohol-derived acetaldehyde. In another study, alcohol consumption was also associated with increased DNA damage as measured by the COMET assay (26). In contrast, supplementation with moderate amounts of red wine decreased leukocyte 8-oxodGao and increased total antioxidant capacity.
in a group of volunteers consuming a Mediterranean diet (27), and still other studies showed no correlation at all between alcohol intake and 8-oxodGuo in lymphocytes (15) or between alcohol drinking and 8-oxodGuo in leukocytes from buffy coat (28). The discrepancies between these previously reported findings might be due to differences in the methods of 8-oxodGuo measurement, the types of alcoholic beverages considered and the level and duration of consumption. Furthermore, differences in genetic susceptibility—for example, polymorphisms in aldehyde dehydrogenase (14) or DNA repair genes (29,30)—might also explain some of the discrepancies.

There are arguments in favour of wine, especially red wine, being superior to other alcoholic drinks in protecting against oxidative damage, because of a high content of polyphenolic compounds (flavonoids and non-flavonoid components) (31). These phytochemicals are antioxidants and free radical scavengers and inhibit lipid peroxidation processes (32). Administration of wine complex polyphenols and tannins have been shown to reduce 8-oxodGuo in rat liver DNA (33) and phenolic substances in red wine have demonstrated a potent antioxidant effect on low-density lipoproteins (34). Interestingly, however, our results indicate an inverse correlation...
between level of 8-oxodGuo and alcohol intake, regardless of the type of alcoholic beverage. This suggests a possible protective effect due to alcohol itself, although it cannot be ruled out that such effect was due to other anti-oxidant components present in these different beverage types.

To our knowledge, this cross-sectional study is the largest conducted so far, and it shows a remarkable consistency of findings across study centres, both at the level of the individual and at the group level. However, although our findings persisted after adjustments for the possible confounding effects of total fruit and vegetable consumption and plasma carotenoid levels, our study still has all the usual limitations of cross-sectional studies, in that our results could still be confounded by other, unidentified factors. Such confounding effects may only be overcome entirely by randomized intervention studies, where subjects are given different amounts and types of alcoholic beverages for various periods of time. Our unexpected findings may provide a strong incentive for the conduct of such intervention studies. Without confirmation from intervention studies and before having more detailed insight into the mechanisms of possible effects of moderate alcohol intake on oxidative stress parameters, it would be unwise to advocate the consumption of alcohol to diminish the risk of chronic diseases potentially related to oxidative processes.

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References


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