However, polyphenolics are poorly absorbed, and their plasma concentrations after wine consumption are insufficient to explain the observed increase in antioxidant capacity (3). Red wine consumption is also linked to the acute increase in serum urate concentration that significantly contributes to the observed increase in serum antioxidant capacity (4, 5). However, the exact wine compounds responsible for the increase in urate have not been determined.

For our study, 9 healthy male non-smoking volunteers (25–40 years of age) randomly consumed 4 beverages (3 mL/kg of body weight) in a cross-over design after an overnight fast, over the period of 4 weeks, 1 beverage per week. The following beverages were consumed: red wine, polyphenolics-stripped red wine, ethanol–water solution (water containing 14% ethanol by volume), and water. Beverages were consumed over 5 min. The University of Split School of Medicine’s Ethics Committee approved the study. Blood samples were drawn before and 30, 60, 90, 120, and 180 min after consumption into heparin-containing Vacutainers. We measured plasma antioxidant capacity (as ferric-reducing antioxidant power) (6) and the concentrations of catechin, as a representative of wine polyphenolics (by HPLC coupled with fluorescence detector), and urate (by the uricase method). Removal of polyphenolics was achieved by use of polyvinylpyrrolidone (7) and confirmed by the Folin–Ciocalteau method (8).

Only wine consumption caused an increase in plasma catechin values. In contrast to ethanol and water, consumption of both wine and polyphenolics-stripped wine produced increases in plasma urate concentrations and antioxidant capacity, reaching peak values after 60 min (Fig. 1). Other constituents, administered in the concentrations found in red wine, were subsequently tested to precisely identify the one causing the increased plasma urate concentration and antioxidant capacity. We successfully replicated this increase with a mixture of 8 g/L glycerol in ethanol–water solution (Fig. 1). There was no glycerol effect if ethanol was removed from the solution. Consumption of organic acids (mixture containing 1 g/L each of lactic, malic, tartaric, acetic, and citric acid) or sugars (mixture containing 2 g/L each of glucose and fructose) in ethanol–water solution had no effect.

Our results indicate that the increase in plasma urate after red wine consumption is polyphenolics-independent and that the combination of glycerol and ethanol is responsible for the urate-related increase in plasma antioxidant capacity. This phenomenon could be linked to the finding that significant depletion of ATP and adenine nucleotides (which may indicate that they had been metabolized to urate) occurred only in rat liver slices incubated in a medium containing both glycerol and ethanol (9). The detailed mechanism by which this effect occurs is yet to be elucidated.

Our finding that glycerol and ethanol interact in modulating urate production and the related increase in plasma antioxidant capacity provides a novel experimental direction for clarifying the biological effects of red wine.

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Fig. 1. Changes (in corresponding units and percentages) from initial plasma urate concentrations (□) and antioxidant capacity (■) 60 min after consumption of red wine (RW), polyphenolics-stripped red wine (PSRW), ethanol–water solution (water containing 14% ethanol by volume; ET), water, and a combination of ethanol–water solution and 8 g/L glycerol (ET + glycerol).

Values are the mean (SE; error bars) for 9 participants. equiv., equivalents.
Effect of Delayed Processing on High-Sensitivity C-Reactive Protein

To the Editor:

The concentration of C-reactive protein (CRP) in blood is considered a marker of inflammation; it is also predictive of cardiovascular disease and possibly cancer (1, 2). Although CRP is frequently measured in large studies, the effects of storage conditions and delays in processing on those measurements are largely unknown. In whole blood samples stored at ambient temperature, CRP has been reported to be stable for 6 h (3) and for 3 days (4), and in samples stored on ice it has been reported as stable for 36 h (5). However, in multicenter studies for which analyses are conducted at a single site, specimens are often shipped overnight. It is possible to ship specimens under refrigerated or frozen conditions, but doing so adds to the cost of the study. We therefore assessed the stability of high-sensitivity CRP (hsCRP) in unprocessed whole blood samples shipped overnight at ambient temperature.

This study was part of a larger study of changes in immune and inflammatory markers and quality of life among people staying at an institute that teaches about a raw vegan diet (Hippocrates Health Institute, W. Palm Beach, FL). The protocol of our study was approved by the Institutional Review Board at Columbia University, and informed consent was obtained from all participants before blood sample collection. We obtained 2 fasting blood specimens from each of 21 volunteers. Blood from each participant was collected into two 4-mL serum separator tubes (Becton Dickinson). One specimen from each participant underwent immediate processing. Within 3 h of collection, these specimens were centrifuged at 1200g for 10 min, and the serum was then poured into a microcentrifuge tube. The other specimen from each participant underwent delayed processing. These specimens were shipped overnight at ambient temperature and were centrifuged ~28 h after collection at 1510g for 10 min. The serum was then poured into microcentrifuge tubes. All serum specimens were then stored at −80 °C. The hsCRP concentrations in the immediate and delayed processing samples were measured simultaneously, in duplicate, with a standardized ELISA, according to the manufacturer’s directions (Life Diagnostics, Inc.; detection limit, 0.1 mg/L). Absorbance was read at 450 nm with a microtiter well reader. hsCRP values <0.1 mg/L were recorded as 0.09 mg/L.

The results did not follow a gaussian distribution; we therefore used nonparametric statistical methods (SPSS, Ver. 10.0.5). We log-transformed the data to determine the intraclass correlation coefficient. A two-sided P value <0.05 was considered significant.

As is evident in Fig. 1, hsCRP concentrations were increased with delayed processing in 67% of the participants. The greatest variability occurred with values <2.5 mg/L; in a comparison of immediate vs delayed processing for hsCRP values >2.5 mg/L, 6 of 8 differed by <18%. The median (interquartile range) hsCRP concentration was 1.63 (0.55–4.33) mg/L in the group of specimens processed immediately and 1.49 (0.69–4.82) mg/L in the delayed processing group, a 9% decrease in the latter group. A Wilcoxon signed-ranks test showed a significant difference between immediate and delayed processing (P = 0.037); however, the Spearman correlation coefficient was 0.97 (P < 0.01) and the intraclass correlation coefficient was 0.94 (P < 0.01).

In summary, the correlation between hsCRP for immediate and delayed processing was strong; however, most values were higher after delayed processing. The lower median hsCRP with delayed processing likely reflects the relatively wide variation in hsCRP results between processing procedures when the values are low. The impression associated with hsCRP processing, ~3.5%–7.0% (4), is smaller than the 9% difference found in this study. In one of the previous studies, mean serum CRP concentrations in 15 specimens analyzed after a 6-h delay in processing were 6% lower than those in the samples processed immediately (3). In another study with 5 participants, the concentrations in EDTA–whole blood specimens processed after a 3-day delay differed by <10%, with no mention of direction (4). In a third study of 17 participants, mean concentrations in hiraparinized specimens stored on ice were 2% higher after a 36-h delay in processing (5).

![Fig. 1. Concentrations of hsCRP in samples analyzed after immediate and delayed centrifugation.](Image)

Each data point represents one participant. The solid line represents the mean percentage difference; the dashed lines represent the mean percentage difference ± 2 SD. Y-axis values were calculated by use of the formula: [(delayed − immediate centrifugation results)/immediate centrifugation results] × 100.
In conclusion, when clotted whole blood samples are shipped at ambient temperature and serum processing is delayed for 28 h, there may be small changes in hsCRP concentrations measured with ELISA, particularly with lower values. These results should be confirmed in a larger sample and for hsCRP measured with automated methods.

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References

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