Acute, food-induced moderate elevation of plasma uric acid protects against hyperoxia-induced oxidative stress and increase in arterial stiffness in healthy humans

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ABSTRACT

We examined the effects of acute, food-induced moderate increase of plasma uric acid (UA) on arterial stiffness and markers of oxidative damage in plasma in healthy males exposed to 100% normobaric oxygen. Acute elevation of plasma UA was induced by consumption of red wine, combination of ethanol and glycerol, or fructose. By using these beverages we were able to separate the effects of UA, wine polyphenols and ethanol. Water was used as a control beverage. Ten males randomly consumed test beverages in a cross-over design over the period of 4 weeks, one beverage per week. They breathed 100% O2 between 60th and 90th min of the 4-h study protocol. Pulse wave augmentation index (AIx) at brachial and radial arteries, plasma antioxidant capacity (AOC), thiobarbituric acid-reactive substances (TBARS), lipid hydroperoxides (LOOH) assessed by xylenol orange method, UA and blood ethanol concentrations were determined before and 60, 90, 120, 150 and 240 min after beverage consumption. Consumption of the beverages did not affect the AIx, TBARS or LOOH values during 60 min before exposure to hyperoxia, while AOC and plasma UA increased except in the water group. Significant increase of AIx, plasma TBARS and LOOH, which occurred during 30 min of hyperoxia in the water group, was largely prevented in the groups that consumed red wine, glycerol + ethanol or fructose. In contrast to chronic hyperuricemia, generally considered as a risk factor for cardiovascular diseases and metabolic syndrome, acute increase of UA acts protectively against hyperoxia-induced oxidative stress and related increase of arterial stiffness in large peripheral arteries.

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1. Introduction

In contrast to chronic hyperuricemia, generally considered as a risk factor for cardiovascular and renal disease, hypertension and metabolic syndrome [1,2], acute increase of plasma uric acid (UA) may induce various beneficial effects to human subjects. Administration of UA increases plasma antioxidant capacity [3], reduces exercise associated oxidative stress in healthy subjects [4,5] and restores endothelial function in patients with type 1 diabetes and regular smokers [6]. UA, as the most abundant aqueous antioxidant, accounting for up to 60% of plasma antioxidative capacity [7], may involve different mechanisms of action. It is a free radical scavenger [8] which stabilizes vitamin C in serum, mostly due to its iron chelating properties [9,10], and quenches peroxynitrite, a potentially harmful oxidant, resulting in formation of a stable nitric oxide (NO) donor in vitro [11]. At concentrations close to physiological levels in humans, UA prevents hydrogen peroxide-induced inactivation of extracellular superoxide dismutase (ecSOD), an enzyme that scavenges superoxide anions (∙O2−) [12]. Also, it has been suggested that UA counteracts oxidative damage related to atherosclerosis and aging in humans [13]. Taken together, these findings imply that UA could act beneficially in preserving vascular function, both under physiological and pathological challenges.

One of such challenges is hyperoxia, which causes vasoconstriction [14,15] and acutely increases arterial stiffness, as manifested by elevation of augmentation index (AIx), in healthy subjects [16]. Oxygen is widely used in the treatment of a diverse range of acute medical conditions. However, it is often administered empirically, without prior knowledge of arterial oxyhaemoglobin saturation. As a result, patients may be exposed to significant periods of hyperoxia. A potential mechanism by which hyperoxia may affect vascular reactivity is the generation of reactive oxygen species (ROS). For example, it has been demonstrated in vitro that ∙O2− produced during hyperoxia reacts rapidly with NO [17], thereby decreasing...
its bioavailability. ROS may also affect the redox state of tetrahydrobiopterin (Bh4), impairing its function as an essential cofactor for endothelial NO synthase [18]. Hyperoxia may also interact with nitrosylated haemoglobin and/or thiol compounds which serve as vehicles for circulatory NO transport. Release of NO from these compounds is likely inversely related to blood O2 tension [19].

Application of antioxidants has been proposed as a therapeutic strategy against hyperoxia-induced ROS. Vitamin C prevented decrease of coronary flow velocity and increase of coronary resistance in patients with ischemic heart disease after hyperoxic challenge [20]. In another study, vitamin C prevented hyperoxia-mediated vasoconstriction and impairment of endothelium-dependent vasodilation [21]. The effects of UA on the hyperoxia-induced oxidative stress and vascular activity have not been tested in human subjects.

We examined the effects of acute, moderate increase of plasma UA on arterial stiffness and markers of oxidative damage in plasma in healthy human subjects exposed to 100% normobaric oxygen. Acute elevation of plasma UA was induced by consumption of red wine, combination of ethanol and glycerol, or fructose. We have previously shown that the increase in plasma antioxidative capacity after red wine consumption is mainly uric acid-related and that ethanol and glycerol were the compounds in red wine responsible for such acute elevation of UA [22,23]. Also, it has been reported that high fructose intake results in acute increase in plasma UA due to accelerated degradation of purins [24]. By using red wine, ethanol + glycerol and fructose, we were able to separate the effects of UA elevation from the activity of wine polyphenols and ethanol.

2. Materials and methods

2.1. Chemicals

Trolox, 2,4,6-tri(2-pyridil)-s-triazine (TPTZ), ascorbic acid, ferric chloride hexahydrate, hydrochloric acid, perchloric acid, 1,1,3,3-tetrametoxypropane, sodium acetate trihydrate and glacial acetic acid were purchased from Sigma-Aldrich Chemie (Steinheim, Germany), ethanol (70%, v/v) from the winemaking factory (Segesta, Croazia), xylene-orange (XO) and ammonium iron (II) sulphate from Merck KgaA (Darmstadt, Germany), thiobarbituric acid (TBA) from Fluka Chemie GmbH (Buchs, Switzerland), tricloracetic acid (TCA) and hydrogen peroxide (30%, w/v) from Panreac Quimica S.A.U (Barcelona, Spain). Deionized (Milli Q) water was used for preparation of all solutions and reagents.

2.2. Preparation of beverages

Red wine (Vinagra, vintage 2005, 13%, v/v, ethanol) was provided by the Bric winery, Slovenia. Ethanol + glycerol solution was prepared by diluting 70% ethanol solution with tap water to 13% (v/v), after which glycerol was added up to 8 g/L. Fructose solution was prepared by diluting 80 g of fructose in 300 mL of tap water.

2.3. Plasma antioxidant capacity (AOX)

The antioxidant capacity (AOX) of plasma was measured by ferric reducing antioxidant power (FRAP) assay. In this assay, antioxidants are evaluated as reductants of Fe3+ to Fe2+, which is chelated by TPTZ to form a Fe2+-TPTZ complex absorbing at 593 nm [25]. Absorbance was monitored by UV–vis spectrophotometer (Specord 200, Analytik Jena Inc., Jena, Germany), equipped with a six-cell holder and a thermostatically controlled bath. All measurements were done in triplicate. Results were compared with a standard curve prepared daily with different concentrations of Trolox, a water-soluble analog of Vitamin E, and expressed as micromolar Trolox equivalents.

2.4. Thiobarbituric acid-reactive substances (TBARS)

TBARS assay is based on reaction of malondialdehyde (MDA), one of end products of lipid peroxidation, with TBA [26]. To correct for background absorption, the absorbance values at 570 nm were subtracted from those at 532 nm, which represent the absorption maximum of the TBA:MDA adduct [27]. Absorbances were monitored by the above mentioned UV–vis spectrophotometer. All measurements were done in triplicate. Results were compared with a standard curve prepared daily with different concentrations of MDA and expressed as micromolar MDA equivalents.

2.5. Plasma lipid hydroperoxides (LOOH)

Plasma lipid hydroperoxides were measured by ferrous oxidation in xylene-orange (FOX) assay. The assay utilizes oxidation of Fe2+ to Fe3+ by lipid hydroperoxides under acidic conditions. Ferric ions interact by the ferric ion indicator, xylene orange, generating a blue-purple complex. To correct for background absorption, the absorbance values at 700 nm were subtracted from those at 570 nm, which represent the absorption maximum of the new generated complex [28,29]. Absorbance measurements were done in triplicate. Results were compared with a standard curve prepared daily with different concentrations of H2O2 and expressed as micromolar H2O2 equivalents.

2.6. Plasma and blood biochemical measurements

Plasma uric acid concentrations were determined by automatic analyzer Olympus AU 600 (Olympus Michima Co. LTD, Shizouka, Japan) and enzymatic laboratory kit. Blood-ethanol concentration (BEC) was measured by Shimadzu 2010 gas chromatography with headspace and flame ionization detector (FID). Ultra-pure grade helium was used as the carrier gas at a flow rate of 11.70 mL/min. The chromatographic column was RTX-BA2 (Fused Silica, 30 m, and 0.53 mm i.d., with a film thick 0.20 μm). Injection temperature was 200 °C, column conditions 3 min at 60 °C, FID 200 °C.

2.7. Measurements of arterial stiffness

The augmentation index (Alx) is an indirect marker for arterial stiffness and a direct measure of wave reflection [30,31]. In principle, the Alx corresponds to the pressure difference between initial systolic (P1) and reflected wave (P2) in a relation to the pulse pressure (PP). It is calculated on the basis of the formula: Alx (%) = [(P2-P1)/PP] × 100 [32]. In this study we measured Alx non-invasively by Sphygmocor (AtCor Medical, Sydney, Australia) and recently validated instrument Arteriograph (TensioMed™ Kft, Budapest, Hungary) [32]. Sphygmocor determines Alx based on the principle of annulation tonometry on radial artery using strain gauge transducer placed on the tip of a pencil-type tonometer [33]. Arteriograph determines Alx by oscillometric method that measures periodic pressure changes in the inflated cuff induced by fluctuations in pulsatile pressure in the brachial artery beneath [32]. It was shown that Alx values measured by both methods highly correlate [32].

2.8. Study design

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University of Split School of Medicine. All subjects gave written consent prior to their participation in the study. Ten healthy male volunteers, aged 20–35 years (mean body mass index of 24.2 ± 1.8 kg/m2) were normolipidemic (total cholesterol < 5 mmol/L, LDL < 3 mmol/L, HDL > 1 mmol/L, triglycerides < 1.7 mmol/L), nonsmokers, taking...
no medications and had no history of alcohol abuse. Subjects were asked to abstain from exercise, fruits, vegetables, dietary supplements, tea, alcoholic beverages, and caffeine- or theobromine-containing foods for 24 h before each visit. After an overnight fast, the subjects randomly consumed one of four test beverages in a cross-over design over the period of 4 weeks, one beverage per week (3 mL/kg body wt, 210–285 mL total amount for each beverage per subject, depending on the subjects body weight). The following beverages were consumed: (1) red wine, (2) ethanol + glycerol solution, (3) fructose solution and (4) water (control).

All experiments were carried out in a quiet, temperature-controlled room maintained around 24 °C and were started at 8 a.m. Subjects rested for 15 min in a supine position before measurement and remained in that position through the 4-h study protocol, abstaining from any food or additional beverages. They breathed 100% O2 for 30 min between 60th and 90th min of the protocol. Oxygen was administered at the rate of 15 L/min via non-rebreathing mask.

A venous cannula (22 G) was inserted into large subcutaneous vein in the left antecubital fossa to allow blood sampling. Blood samples were collected into heparin and sodium fluoride/potassium oxalate vacutainers (Becton Dickinson, UK), immediately before (baseline) and 60, 90, 120, 150 and 240 min after beverage consumption. Plasma TBARS, LOOH, AOC and uric acid values and blood ethanol concentration were analyzed immediately after sampling. All biochemical parameters were measured by persons blinded for sample source regarding subject and beverage consumed. At the same six time points, Alx, heart rate (HR), systolic, diastolic and mean arterial pressures were determined.

2.9. Statistics

Data are expressed as mean ±S.E.M. Statistical analyses were performed using Statistica 6.0 (StatSoft Inc., Tulsa, USA). Two-way ANOVA for repeated measures was used to evaluate beverage-dependent changes in Alx, heart rate (HR), mean arterial pressure (MAP) and plasma AOC, TBARS, LOOH and uric acid values in plasma and ethanol in the blood. When statistical significance was reached by ANOVA (P < 0.05), Bonferroni test was used for the post hoc analysis.

3. Results

Baseline heart rate (HR) values in the examinees prior to consumption of red wine, ethanol + glycerol, fructose and water were 56.0 ± 1.7, 57.0 ± 1.7, 57.6 ± 2.7 and 58.0 ± 1.9 beats/min, respectively. During the experimental protocol no beverage-dependent changes in HR values occurred (Fig. S1).

Similarly, baseline MAP values in red wine, ethanol + glycerol, fructose and water groups were 80.1 ± 2.0, 79.8 ± 1.9, 81.6 ± 1.0 and 82.7 ± 2.1 mmHg, respectively, and did not change during the experiment (Fig. S2).

Initial augmentation index (Alx) values in the red wine, ethanol + glycerol, fructose and water groups obtained by Arteriograph were −60.8 ± 2.9, −62.5 ± 1.9, −61.0 ± 4.2 and −61.7 ± 2.9% and did not differ either among each other or in comparison with baseline Alx values obtained by SphygmoCor that were −58.3 ± 1.5, −62.0 ± 1.9, −61.8 ± 2.6 and −57.6 ± 1.6%, respectively. Consumption of beverages did not induce changes in either TBARS or LOOH values during 60 min, before exposure to hyperoxia. Administration of 100% normobaric oxygen for 30 min caused a marked increase of TBARS and LOOH in the water (control) group in contrast to other test beverages (Fig. 2).

Baseline plasma uric acid (UA) values were 359 ± 14, 358 ± 18, 348 ± 20 and 339 ± 13 μmol/L for red wine, ethanol + glycerol, fructose and water groups, respectively. Consumption of test beverages, except water, resulted in significant increase in plasma UA values (Fig. 3). Exposure to hyperoxia did not cause further changes in plasma UA values.

Baseline plasma antioxidant capacity (AOC) values were 637 ± 20, 655 ± 22, 655 ± 17 and 632 ± 23 μmol/L Trolox equivalent (TE) for red wine, ethanol + glycerol, fructose and water, respectively. Consumption of all test beverages, except water, induced significant increase in plasma AOC (Fig. 4). Exposure to hyperoxia induced an additional temporary elevation of plasma AOC that was similar in all groups.

Baseline blood ethanol concentration (BEC) values were zero in all groups. Ethanol + glycerol solution and red wine induced a significant and similar increase in BEC values (Fig. S3).
Changes in plasma uric acid (UA) values after consumption of the test beverages and oxygen administration. Plasma UA values were determined before and 60, 90, 120, 150 and 240 min after consumption of red wine (●), ethanol + glycerol (▲), fructose (●) and water (■). Data are mean changes (△) ± S.E.M. (μmol/L MDA and LOOH) = 10 per beverage. Baseline plasma UA values were 359 ± 14, 358 ± 18, 348 ± 20 and 339 ± 13 μmol/L for red wine, ethanol + glycerol, fructose and water, respectively. △P < 0.05 water vs. all other beverages (Two-way ANOVA for repeated measures).

Fig. 2. Changes in biochemical markers of oxidative stress, thiobarbituric acid-reactive substances (TBARS) (panel A) and plasma lipid hydroperoxides (LOOH) assessed by xenon orange method (panel B) after consumption of the test beverages and oxygen administration. TBARS and LOOH values were determined before and 60, 90, 120, 150 and 240 min after consumption of red wine (●), ethanol + glycerol (▲), fructose (●) and water (■). Data are mean changes (△) ± S.E.M. (μmol/L MDA and μmol/L H₂O₂) = 10 per beverage. Baseline TBARS values were 0.149 ± 0.016, 0.171 ± 0.008, 0.162 ± 0.011 and 0.159 ± 0.008 μmol/L MDA and LOOH values were 10.5 ± 1.5, 12.2 ± 1.1, 12.4 ± 1.4 and 9.8 ± 1.4 μmol/L H₂O₂ for red wine, ethanol + glycerol, fructose and water, respectively. △P < 0.05 water vs. all other beverages (Two-way ANOVA for repeated measures).

4. Discussion

In the present study we demonstrated protective effects of acute increase of uric acid (UA) against hyperoxia-induced oxidative stress and increase of arterial stiffness in large peripheral arteries. Elevation of UA largely prevented hyperoxia-induced increase of AIx, as well as the increase in plasma TBARS and LOOH assessed by xenon orange method, markers of oxidative stress.

Original feature of this study is a non-invasive approach to inducing acute increase of plasma UA, by consuming red wine, ethanol + glycerol and fructose solutions, generally safe substances that are regularly consumed in everyday life. By combining these solutions we were able to separate the effects of UA, wine polyphenols and ethanol. The significance of metabolic interference of fructose with purine nucleotides metabolism has been proven in the study by Lotito and Frei [34], which showed that the increase in human plasma antioxidant capacity (AOC), after apple consumption, is due to metabolic effects of fructose on UA.

In our previous study we have showed that the increase in plasma AOC after red wine consumption is also mainly mediated by the increase of plasma UA and to a lesser degree by the wine polyphenolics, as determined by the increase of plasma levels of catechin, the major representative of red wine polyphenolics [22]. Although we did not measure presence of wine phenolics in plasma in the present study, our results are in complete agreement with the previous findings, as red wine induced increase in plasma antioxidative capacity similarly to ethanol + glycerol and fructose solutions (Fig. 4), while it caused smaller increase in plasma UA concentration (Fig. 3). This indicated that, beside UA, the red wine-induced increase in plasma antioxidative capacity in humans is also mediated by additional mechanisms such as wine polyphenolics [35,36]. It should be kept in mind, however, that not only polyphenols absorbed to blood but also unabsorbed polyphenols that remained in gastrointestinal tract may affect plasma antioxidant activity. Through their local antioxidant activity, they may spare other antioxidants from oxidation and thereby influence the whole organism and plasma concentration of various antioxidants [37,38]. Relative contribution of these local actions of polyphenols to their antioxidative effects at the level of whole organism cannot be estimated at present.

The same reasoning may equally be applied to the effects of red wine on arterial stiffness. Red wine, similarly to ethanol + glycerol and fructose solutions, prevented hyperoxia-induced increase in AIx (Fig. 1), in spite of the difference in corresponding plasma UA levels, indicating specific role of wine polyphenols in the observed effects. Similar beneficial effect of red wine intake in reducing arterial stiffness was also observed in patients with coronary artery disease [39] and in healthy subjects after cigarette smoking [40].
On the other side, obtained beneficial effects of acute plasma UA increase are also in line with the studies in which increase of plasma UA after oral administration of its precursor inzine monophosphate (IMP) [5], or after intravenous administration of UA [4,6], resulted in elevation of antioxidant capacity, reduction of oxidative stress and improvement of endothelial function. It is important to notice that beneficial effects of UA in our study were induced by moderate, approximately 15% elevation in UA levels, in contrast to aforementioned studies in which increase of UA was between 60 and 100% [4–6]. Expectedly, such moderate increase in plasma UA did not alter basal AIx, as much higher acute increase in plasma UA concentration, in the study by Waring et al., also did not affect vascular function in healthy subjects [6]. The fact that acute increase of UA in such a wide range of concentrations similarly affected vascular function implies possibility of a threshold in the UA-mediated prevention of vascular dysfunction, above which no further benefits are observed.

Our results also actualize differential role of acutely increased UA in contrast to chronic hyperuricemia. Namely, over the last decade, epidemiological studies have indicated that plasma UA is an independent risk factor for cardiovascular disease, especially in patients with diabetes, hypertension, or heart failure [1,2]. Patients with hypertension and hyperuricemia have increased risk of coronary artery disease (CAD) or cerebrovascular disease, compared with patients with normal UA levels [41]. Moreover, increased serum UA is highly predictive of mortality in patients with heart failure or CAD and of cardiovascular events in patients with diabetes [42,43]. Although these findings strongly support the perception that elevated plasma UA plays a causal role in the development of cardiovascular disease, mechanisms that might be involved have not been clearly established. In several recent studies partial explanations have been offered for paradoxical role of UA related to cardiovascular diseases. Lippi et al. in their critical review describe how antioxidant compounds such as UA, may become pro-oxidants under different pathophysiological conditions when UA is present in supranormal levels [44].

This was further elaborated in the review by Strazzullo and Puig who suggested that in general population samples at relatively low risk of cardiovascular diseases, UA is a very weak predictor of cardiovascular morbidity and mortality, once the effect of known confounders is accounted for [42]. Conversely, UA appears to be a significant independent predictor of cardiovascular disease in hypertensive patients and in certain subjects at high or very high cardiovascular risk, namely diabetics, stroke survivors, patients with congestive heart failure and those with angiography proven coronary heart disease. The net discrepancy between the findings in relatively healthy persons and those in high-risk individuals raise the question of possibly different meaning of excess UA levels in different circumstances. This concept is best illustrated in two pathophysiological conditions that are very frequent in high-risk individuals: local ischemia and obesity.

The pro-oxidant and pro-inflammatory actions attributed to UA could be largely the result of the conversion of xanthine dehydrogenase to xanthine oxidase and of the consequent accumulation of reactive oxygen species (ROS) which occurs in parallel with UA production as an effect of ATP degradation under ischemic conditions. In this case, the ROS by-production might cause the inflammatory reaction and the arterial wall damage which have been attributed to excess of UA [42].

Obesity associated oxidative stress in adipose tissue has recently been recognized as an important cause of insulin resistance and cardiovascular disease. It was shown that UA may stimulate an increase in NADPH oxidase activity and related production of ROS in adipocytes. It is followed by redox-dependent signaling and oxidative modifications of proteins and lipids. Therefore, hyperuricemia, as a persistent condition associated with obesity, may be a significant factor contributing to obesity related oxidative stress [45].

On the other side, it should be kept in mind that supranormal plasma UA levels might as well indicate compensatory response of the organism to the increased oxidative stress related to different pathophysiological challenges. Therefore, the role of an increased UA as a causal, compensatory, or coincidental risk factor in patients with CAD remains unclear [46,47]. In that regard, it should be noticed that acute actions of established cardiovascular risk factors, such as high-fat meals, hyperhomocysteinemia, or cigarette smoking, cause immediate impairment of vascular function [48–50], in contrast to acutely increased plasma UA.

In addition to the increase of LOOH and TBARS, markers of oxidative stress, hyperoxia also caused additional increase in plasma antioxidative capacity in all study groups (Fig. 4). Similar phenomena were observed in rats exposed to hyperoxia [51] and in humans after exercise [52], with simultaneous increase in antioxidative capacity and oxidative stress markers. The underlying mechanism of this effect is unclear. It was proposed that hyperoxia induces redistribution of proteins and low-molecular-weight antioxidants from lungs to systemic bloodstream [51]. Indeed, in several studies elevation of different antioxidants was observed after exposure of hyperoxia: tissue glutathione and vitamin C levels in guinea pigs [53], tissue glutathione and vitamin E levels in mice [54] and glutathione levels in fetal and adult human skin cells [55]. The identification of the antioxidants responsible for the increase of plasma antioxidative capacity after exposure to hyperoxia in our experimental conditions remains to be determined.

As a potential limitation of this study, it should be mentioned that methodology to evaluate oxidative damage to lipids does not represent the “gold standard” for in vivo studies. However, we used two complementary methods (TBARS and LOOH assessed by xylene orange), which results closely correlated and were in agreement with the kinetics of uric acid and total plasma antioxidative capacity. Hence, methodology used and obtained results may be acceptable as reasonably convincing and reliable.

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Appendix A. Supplementary data


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