METHOD OF ASSESSMENT OF ANTIOXIDANT STATUS IN VIVO

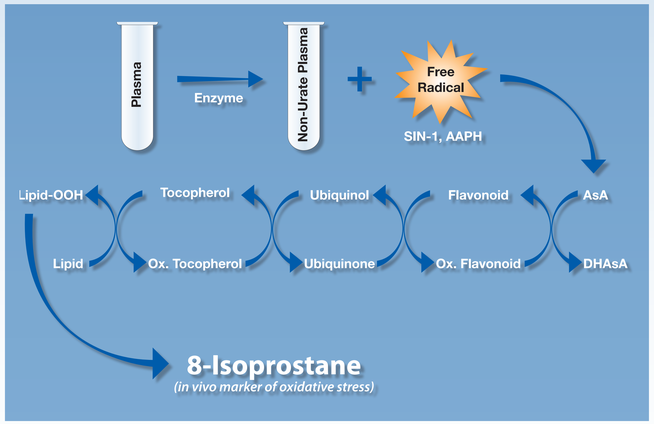
Alexander Rabovsky, John Cuomo, Natalie Eich

Department of Research & Development, USANA Health Sciences, Salt Lake City, UT, USA

INTRODUCTION

Oxidative stress is associated with a variety of chronic degenerative diseases, including cancer, diabetes, cardiovascular diseases, and Alzheimer's disease. An imbalance of oxidants and antioxidants within the human body, in which either oxidants are high or antioxidant protection is low, will lead to a state of oxidative stress. Therefore, the measurement of the antioxidant status of biological fluids could be used as an early warning sign of possible disease onset.

A method has been proposed to measure plasma antioxidant status. This method, plasma antioxidant reserve (PAR), involves two steps. The first step is free radical oxidation of plasma ex vivo and the second step is the measurement of markers of lipid peroxidation, 8-isoprostanes. A third optional step, the enzymatic removal of uric acid via uricase prior to oxidation has also been evaluated and allows a direct measurement of antioxidant status, rather than activity due to uric acid. With this method we are able to identify potential imbalances in antioxidant protection even before clinical symptoms of disease exist.



MATERIALS AND METHODS

Fifteen healthy human subjects volunteered to participate in this study (9 males, 6 females). The average subject was 32 ± 9.9 years old, was 69 ± 3.9 inches tall, and weighed 168 ± 20.4 pounds.

Blood was collected after an overnight fast. Each participant was then given 675 mg of ascorbic acid. Four hours later, a second plasma sample was drawn. This procedure was repeated on separate days with the same subjects supplementing with 1000 IU's vitamin E (USANA Health Sciences E Prime™ capsules), green tea extract, grape seed extract, or quercetin. All supplements were provided by USANA Health Sciences and dosed on an equal weight basis.

Plasma Antioxidant Reserve (PAR)

Each plasma sample was analyzed in duplicate; one set was treated with enzyme mixture (catalase & uricase) and the other set did not receive any treatment with enzyme mixture. Samples treated with enzyme were thoroughly mixed and incubated at 25°C for 10 min following addition of enzyme. Both sets of samples were treated with SIN-1 chloride (0.2 mmol) and were incubated at 37°C for 4 h with shaking. Plasma 8-isoprostanes were measured following incubation with SIN-1 using an ELISA kit (8-iso Prostaglandin F_2 Kit, Cayman Chemical, Ann Arbor, MI).

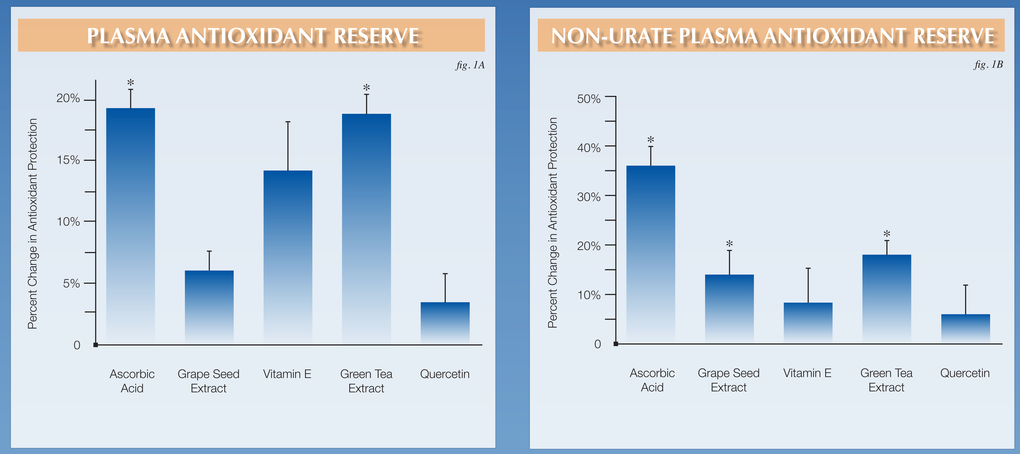


Figure 1. Percent change in antioxidant protection (as measured by percent decrease in induced 8-isoprostane concentration) after supplementation with 5 antioxidants. A) Induced 8-isoprostane formation by oxidation with SIN-1 in the presence of 5 different antioxidants. B) Induced 8-isoprostane formation after the removal of uric acid and then oxidized with SIN-1 in the presence of 5 different antioxidants. Error bars are ± standard error of the mean. P values were calculated from absolute values at baseline and 4 h after supplementation by a paired t-test. *P<0.05

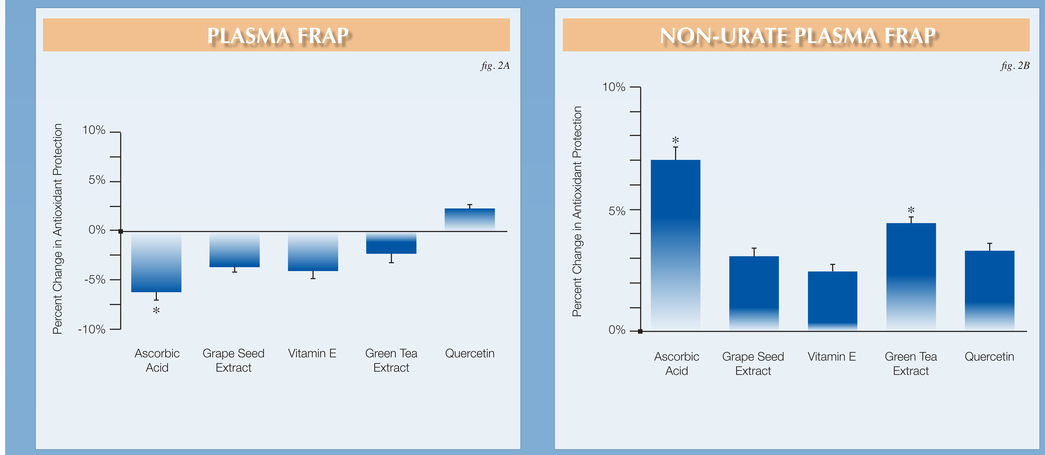


Figure 2. Percent change in antioxidant protection (as measured by an increase in ferric reducing antioxidant power of plasma or FRAP) after supplementation with 5 different antioxidants. A) Plasma antioxidant capacity as determined by FRAP. B) Plasma antioxidant capacity determined by FRAP assay after the removal of uric acid for 5 antioxidants. Error bars are ± standard error of the mean. P values were calculated from absolute values at baseline and 4 h after supplementation by a paired t-test. *P<0.05

Ferric Reducing Antioxidant Power of Plasma (FRAP)

For comparison FRAP was also determined for plasma samples. In brief, samples were either diluted 1:2 with 0.15M NaCl or with enzyme mixture to simulate the conditions of the PAR assay. A reagent solution containing 0.8 mmol/l TPTZ and 1.7 mmol/l $FeCl_3$ was added to each sample. The samples were incubated at 37°C for 15 min and the absorbance measured at 593 nm (Molecular Devices, Spectramax 340 pc).

RESULTS

In each of the 5 cases, there were more isoprostanes formed in the fasting sample than in the sample taken after ingesting the antioxidants. Ascorbic acid supplementation decreased isoprostane formation from 163±9.6 to 131±5.6 pg/ml (P<0.001). Vitamin E, grape seed extract, green tea extract, and quercetin all had similar effects on isoprostane levels (Figure 1A). Furthermore, the enzymatic removal of uric acid from plasma prior to oxidation amplified the effect of antioxidant supplementation. After the removal of uric acid, ascorbic acid supplementation reduced isoprostane formation from 344±34.6 to 220±13.1 pg/ml (P<0.0004), a decrease of 36% compared to a decrease of 19% in the presence of uric acid. Again, even with the removal of uric acid the effect was still seen with the other antioxidants (Figure 1B).

In contrast with the results from the PAR assay, the FRAP values measured did not change significantly when antioxidants were consumed (Figure 2A). However, once uric acid was removed there was a slight improvement in FRAP value with ascorbic acid and green tea extract supplementation (from 242±13.5 to 259±18.1 µmol/l, P<0.02 and from 367±13.4 to 382±14.8 µmol/l, P<0.006, respectively) (Figure 2B).

DISCUSSION

Our results demonstrate that the PAR method can be used to show the effect of short-term supplementation for a wide variety of antioxidants. For each of the 5 antioxidants tested, there were more isoprostanes formed in the fasting sample than in the sample taken after ingesting the antioxidants, showing that the ingested antioxidants, in fact, can improve the resistance of plasma to oxidation. Undoubtedly, ascorbic acid was shown to be the most effective antioxidant in preventing isoprostane formation. With the standard FRAP assay an increase in antioxidants should result in an increased FRAP value. However, there was no effect seen when subjects were supplemented with antioxidants when evaluated by the FRAP method. Thus, the new method is both more sensitive and predictable than one of the most commonly used methods.

The enzymatic removal of uric acid is a noteworthy variation in this method compared to other methods used for determining antioxidant status. Uric acid is a major water soluble antioxidant present in blood. Once uric acid is removed the antioxidant protection due to antioxidants present in the blood (such as vitamins, phytochemicals, carotenoids, etc.) can be measured. This method eliminates the need to evaluate uric acid separately from antioxidant capacity. Taken together, and utilizing this novel method, supplementation with antioxidants, vitamins, and phytonutrients can boost one's plasma antioxidant status and possibly decrease the risk for chronic degenerative diseases.

USANA HEALTH SCIENCES