A Novel Assay for Determining Plasma Antioxidant Capacity

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An imbalance of oxidants and antioxidants within the human body (in which oxidants are high or antioxidant protection is low) leads to a state of oxidative stress. Oxidative stress is associated with a variety of chronic degenerative diseases.

Measurement of the antioxidant status of biological fluids could be used as an early warning sign of possible oxidative stress onset if a simple, reliable method were available for such a measurement. Unfortunately, while several methods can be used to estimate antioxidant status, questionable sensitivity, unreliability, non-reproducible results and procedural difficulties hamper all. In addition, even for the best test (8-isoprostanes), no significant elevation of the measured value occurs until pathological disease is already evident. In other words, while this test can be used to confirm a state of oxidative stress when a subject already has a clinically diagnosed disease, it is of little value as a predictive tool.

In this paper, we describe a novel method for determining overall plasma antioxidant capacity in a sample of human plasma. Our method consists of the following steps:

1. Isolation of a sample of blood
2. Centrifugation of the blood sample to obtain plasma
3. Introduction of a source of free radicals which induces oxidation of the lipoproteins in the plasma
4. Measurement of the markers of lipoprotein oxidation

A second variation in this method is to remove uric acid from the plasma sample prior to introduction of the free radical source. This is done enzymatically with the addition of uricase. Uric acid is a major water-soluble antioxidant present in blood. Once uric acid is removed, the antioxidant protection of other antioxidants in the blood (vitamins, phytochemicals, carotenoids, etc.) can be measured. Taken together, the method can measure total antioxidant capacity of blood as well as non-urate antioxidant capacity.

This method is extremely sensitive to the addition of antioxidants, vitamins, and phytochemicals present in blood and plasma as a result of oral ingestion of supplements. In one experiment, samples of blood were collected from volunteers after an overnight fast. After fasting blood samples were taken, each subject ingested 1200 mg of vitamin C (from 2 tab-

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lets of USANA Poly C). Four hours later, a second blood sample was taken. Isolated plasma from each sample was then mixed with a SIN-1 solution (a free radical source), incubated for 4 hours at 37° C, and 8-isoprostanes were determined by standard protocols with an ELISA kit. A second sample of plasma (at baseline and 4 hours after ingesting vitamin C) was treated with uricase prior to treatment with SIN-1 solution as described above. For comparison, a FRAP (Ferric Reducing Antioxidant Power) assay, one of the most common assays of antioxidant capacity, was also run on the same plasma samples. In a similar manner, the same subjects were given 1000 IU vitamin E, 600 mg green tea extract, 530 mg grape seed extract, and 600 mg olive fruit extract (Olivol™) on separate days.

Summary data from these experiments is shown in Table 1. In every case, there was a large measurable difference in the amount of isoprostanes formed in the initial fasting sample vs. the sample collected four hours after taking the antioxidant supplement. In each of these five cases, more isoprostanes were formed in the fasting sample than in the sample taken after ingesting antioxidants, showing that orally consumed antioxidants are capable of preventing some level of oxidation. With the standard FRAP assay, an increase in antioxidants should result in an increased FRAP value. However, only one of the five experiments (vitamin C) produced a result consistent with added antioxidants. Thus, the new method is both more sensitive and predictable than existing methods.

In the above examples, SIN-1 (a source of free radicals) was used as the initiator, and isoprostanes were measured as a marker of lipid peroxidation. Other radical initiators and other markers of oxidation may also be used in this application.

Table 1

	PAC (isoprostanes found in pg/mL)				FRAP (µM)			
	Standard		Non-Urate		Standard		Non-Urate	
	Baseline	4 hrs	Baseline	4 hrs	Baseline	4 hrs	Baseline	4 hrs
Vitamin C	74	54	336	190	1011	1041	419	488
(% change)		*-27%*		*-44%*		*3%*		*16%*
Vitamin E	74	66	301	263	1064	1033	453	435
(% change)		*-11%*		*-13%*		*-3%*		*-4%*
Grape Seed Extract	72	68	313	277	999	973	442	446
(% change)		*-6%*		*-12%*		*-3%*		*1%*
Green Tea Extract	74	63	302	277	1055	1058	422	447
(% change)		*-14%*		*-8%*		*0%*		*6%*
Olivol™	78	71	294	267	1055	1039	462	462
(% change)		*-5%*		*-9%*		*-1%*		*0%*

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