

Serum antioxidant status among young, middle-aged and elderly people before and after antioxidant rich diet

Limberaki E¹, Eleftheriou Ph¹, Vagdatli E¹, Kostoglou V², Petrou Ch¹

¹Department of Medical Laboratory Studies, School of Health and Care Professions
Alexander Technological Educational Institute of Thessaloniki, Greece

²Department of Informatics, School of Technological Applications, Alexander Technological Educational Institute of Thessaloniki, Greece

Abstract

Background: The influence of factors such as age, sex, life style and smoking on oxidative stress status of the organism remains unclear. There is evidence that dietary intake of antioxidants is thought to enforce the organism ability to counteract free radicals. Administration of synthetic antioxidants as dietary supplements does not seem to have the same beneficial effect as consumption of the same antioxidants as part of food ingredients. This work focuses on the investigation of age and diet effects on oxidative stress and examines the hypotheses of their significant influence.

Methods: Blood samples of 146 volunteers, were collected and allocated in three age groups. All volunteers completed a questionnaire concerning home and working environmental conditions, special habits and dietary preferences. We implemented a thirty days diet rich in antioxidants in 55 volunteers. Antioxidant activity was estimated before and after the special diet by measuring the influence of serum in oxidation of ABTS by the ferryl myoglobinhydrogen peroxide system.

Results: Our findings showed unexpected lower serum total antioxidant capacity (TAC) in younger people (ages 18–35 yrs) 79%, compared to middle aged and elderly individuals and a large increase 62% in serum TAC of all age-groups after the one-month special diet.

Conclusions: These results imply that a diet rich in antioxidants based on antioxidant rich food consumption and not on single antioxidants administration, can increase the antioxidant status of the organism and offer better health. The total serum antioxidant status increases with age and this fact should be taken into account when TAC is measured in different diseases. Hippokratia 2012; 16 (2): 118-123

Key words: antioxidants, serum, age, diet, cortisol

Corresponding author: Eugenia Limberaki, Department of Medical Laboratory Studies, School of Health and Care Professions, Alexander TEI of Thessaloniki, PO Box 141, 574 00, Thessaloniki, Greece. Tel.: +30 2310 791511, e-mail: evlimper@mls.teithe.gr

Reactive Oxygen Species (ROS) are produced in human organism during the normal aerobic metabolism or due to the action of the macrophages as part of the immune response¹. They are playing a dual role as both deleterious and beneficial species². ROS are normally generated by tightly regulated enzymes, such as Nitric oxide synthase (NOS) and Nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase isoforms. It is known that oxidative stress induces or promotes various diseases³ such as atherosclerosis and heart diseases⁴⁻⁶, cancer⁷, neurodegenerative diseases (Alzheimer disease, Parkinson's disease), hypertension^{8,9}, diabetes mellitus¹⁰ and ageing¹¹⁻¹⁵. It is also argued that oxidative stress constitutes one of the main factors responsible for ischemia/reperfusion injury.

Human organism has developed an antioxidant system¹⁶ to counteract ROS and reduce the damage they cause. The antioxidant system includes various endogenous molecules¹⁷ such as reduced glutathione (GSH), uric acid, bilirubin, ceruloplasmin, ferritin, vitamins such

as ascorbic acid, and tocopherol¹⁸ and a lot of enzymes such as superoxide dismutase, catalase, glutathioneperoxidase¹⁹⁻²¹. Food derived antioxidants are added in the total antioxidant activity of human organism. In these antioxidants we can include polyphenols such as quercetin, tannins and flavonoids present in olive oil, tomatoes, berries, grapes, red wine and beer, coffee, tea and cocoa, lycopene found in red and orange fruits such as tomatoes, carrots, watermelon and antioxidant vitamins found in all fruits and vegetables²². The ten top food sources of antioxidants are coffee, tea, banana, dry beans, corn, red wine, beer, apple, tomato, and potato. Administration of synthetic antioxidants as dietary supplements does not seem to have the same beneficial effect as the consumption of the same antioxidants as part of food ingredients. Moreover, the kind of antioxidants in food, as well as, food preparation procedure and food mixtures used, influences their effectiveness.

The cooperation of all antioxidant components can

provide more protection against ROS than any single compound alone. So, a balanced natural antioxidant rich diet is preferred than a diet rich in a single antioxidant. High concentration of blood antioxidants seems to increase organism's defense against certain diseases and treatment with antioxidants is often proposed as part of the therapy^{23,24}. On the other hand, antioxidant levels may be used as a diagnosis or prognosis indicator in some cases. Measurement of the whole blood antioxidant capacity of the organism may give us valuable biological information about the status of the organism.

At present, there are no consistent perspectives about age-associated change of antioxidant capacity. Certain researchers studied the presentation of catalase, peroxide oxidase, some antioxidant vitamins, lipid peroxidation or protein and DNA oxidation and found increase or decrease of different factors with age²⁵⁻²⁷. In most cases a serum Total Antioxidant Capacity (TAC) increase with age was observed. However, different measurement methods lead to different results. For example, measurement with FRAP method which cannot determine antioxidants with sulfhydryl groups²⁸, showed decrease in TAC with age²⁹. In other studies an increase in TAC with age was found as a secondary observation in patients suffering from cancer disease²⁴.

Factors such as sex, stress, physical activity²⁹⁻³¹ smoking³² and diet³³⁻³⁷ also affect total antioxidant capacity. It has been reported that habits such as smoking and bad diet incline toward increase of oxidative stress, while dietary antioxidant intake can increase total antioxidant capacity³⁸⁻⁴².

Another important factor, which is associated with antioxidant levels, is cortisol, usually referred to as the "stress hormone". Cortisol is involved in organism's response to stress and anxiety. It triggers all metabolic mechanisms leading to production of compounds used as energy sources in emergency conditions. Increased cortisol⁴³ concentrations are measured in blood serum of people under biological or emotional stress, depression, sleep deprivation, fever, hypoglycemia, anorexia nervosa and after surgery⁴⁴. Moreover, increase in cortisol concentration has been associated with decrease in total antioxidant levels⁴⁵. The level of some tissue antioxidants is inversely related to cortisol tissue levels⁴⁶. Antioxidant depletion may result as a consequence of the various pathways activated by cortisol or may be the effect of free radical production during cortisol metabolism. Antidepressant therapy resulting in decrease of cortisol levels also results in the increase of the antioxidant concentration.

The effects of age and diet on antioxidant capacity have not investigated sufficiently up to date in Greece. The present study deals with examining the hypotheses of the statistically significant effects of age and diet in Total Antioxidant Capacity using the 2,2'-azino-bis(3-ethylbenzthiazoline)-6-sulphonic acid (ABTS) method to random samples of Thessaloniki residents. The correlation between cortisol and TAC levels is also examined.

Material and methods

Blood samples

Total blood antioxidants were measured in serum of 146 healthy individuals, 55 male and 91 female, living in the same region and were classified in three groups based on their age; A: 16–35 years (n=65), B: 36–60 years (n=33) and C: 61–90 years (n=48). All individuals were volunteers, residents of Thessaloniki, Greece. The participants were fully informed about the purpose and content of the experiment and their informed consent of participation were obtained. They completed a questionnaire concerning place and type of work, life style, special habits, smoking, diet preferences, medicines and chronic diseases. All subjects had to meet the following criteria: they were no athletes, no pregnant and they had not a metabolic disease.

Sample collection

Blood samples were collected from the cubitus vein, at rest, at 08.00 a.m. After the first blood collection, 55 volunteers of all age groups followed a diet rich in antioxidants (fresh fruits and vegetables, coffee, chocolate, olive oil, honey, red wine, tea, tomatoes) for one month. At the end of the month a second blood sample was collected (2nd blood collection). After one week a third blood sample (3d blood collection) was collected. During this week the volunteers were let to select their diet freely. Detailed dietary records were maintained before the study, during the thirty-day period of antioxidant-rich diet and during the week after the second blood collection. The special diet was rich in different antioxidants, such as vitamin C, E, lycopene flavonoids tannins, carotenoids. All samples were kept at -70°C for maximum one month until measurement.

Measurements of total antioxidant capacity

After studying different methods^{47,48} TEAC (trolox equivalent antioxidant capacity) methodology was used to measure the serum total antioxidant capacity. Antioxidant activity was estimated by the influence of 10 μl of serum in oxidation of 2,2'-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) by the ferryl myoglobin- H_2O_2 system, using the antioxidant assay kit of Sigma. Trolox, the water soluble analogue of vitamin E, obtained from the same company, was used as control. Antioxidant concentration was expressed as trolox equivalents.

Measurement of cortisol

Serum cortisol was measured using the enzyme-linked immunosorbent assay (ELISA) kit of HUMAN. The method is based on competitive interaction of serum cortisol and a cortisol-enzyme conjugated provided by HUMAN for a limited number of monoclonal anti-cortisol antibodies bound to the wells of a microtitre ELISA plate⁴⁹. The amount of the bound hormone-enzyme conjugate is inversely proportional to the concentration of cortisol in the specimen. After removal of unbound conjugate by washing, a substrate solution is added and a blue color develops,

Table 1: Percentage differentiation of total serum antioxidant Capacity (TAC) in different age groups compared to the young group of 16-35 yrs.

Group	Age	n	TAC (mM) \pm SD	p ^a	%
A	16 – 35	65	0.97 \pm 0.14		
B	36 – 60	33	1.74 \pm 0.51	0.000	179%
C	61 – 90	48	1.55 \pm 0.47	0.005	160%

Data are means \pm standard deviation (SD).

The Student's t test has been used for all samples.

The results of all age groups were compared with the results of age group 16-35.

p^a is the significance level.

% increase of TAC compared to the results of age group 16-35.

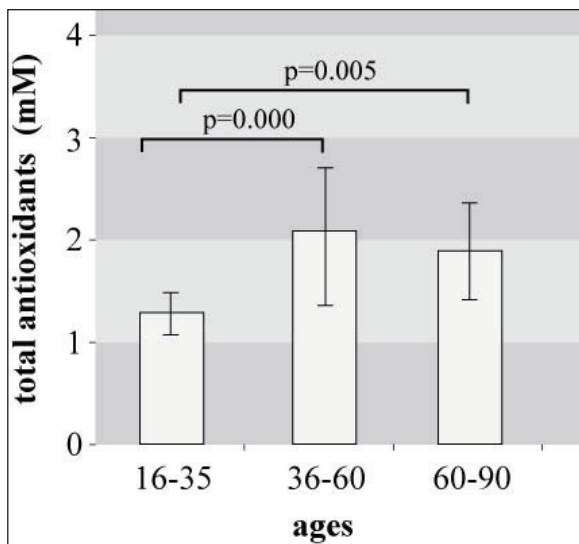


Figure 1: Total antioxidant capacity in blood serum of three age groups.

Significance according to Student's t-test: between age groups 16-35 yrs and 36-60 yrs: p=0.000; between age groups 16-35 yrs and 60-90 yrs: p=0.005.

which changes to yellow by the addition of stop solution. The absorbance is measured at 450 nm using a microtitre plate reader. Concentration of unknown specimens is interpolated from a dose response curve generated by utilizing serum calibrators of known cortisol concentration.

Statistical Analyses

Data were analyzed using the Statistical Package for the Social Sciences (SPSS). Normality of continuous data was determined by Kolmogorov-Smirnov or Shapiro-Wilk normality test. All continuous data following normal distribution are presented as mean \pm standard deviation (SD). Independent-samples t-test was used to compare the blood samples of different age groups while paired-samples t-test was used to determine the effect of extra diet in TAC. A two tailed P value less than 0.05 was considered statistically significant for all comparisons.

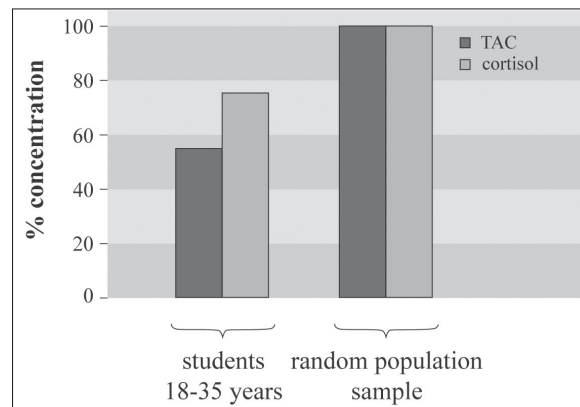


Figure 2: Total antioxidant capacity and cortisol in blood serum of the first age group according to random population.

Results

Experiment 1: Correlation between TAC and age

Factors such as smoking and physical activity which can influence the total antioxidant capacity were similar in all subjects. No significant difference was observed in serum TAC between male and female individuals. Evaluation of the blood antioxidant capacity of the subjects in relation to age revealed a significant difference among young people 16-35 years old and the middle-aged and elderly individuals. (Table 1, Figure 1). The mean values of antioxidant capacity were lower in group A (by 79%) than in the other groups.

Experiment 2: Correlation between TAC and cortisol levels

After the results of the first experiment we tried to understand the lower antioxidant level in younger people. Thus, we measured in this group the serum cortisol levels and compared it with random population. As shown in Figure 2 the antioxidant potential and the cortisol levels were lower in the first age group.

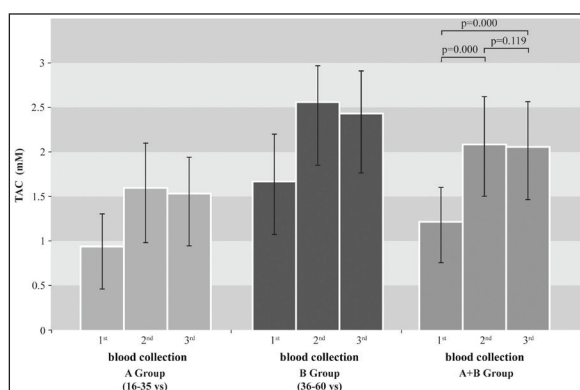
Experiment 3: Correlation between TAC and diet

The correlation of blood antioxidant concentration

Table 2: Serum total antioxidant Capacity and % change after 30day diet full in antioxidants (2nd blood collection) and after 15 day free diet (3d blood collection).

Group	1 st Blood Collection	2 nd Blood Collection		3 rd Blood Collection		
	TAC (mM)	TAC (mM)	% Increase after 1 st blood taking	TAC (mM)	% Increase after 1 st blood taking	% Decrease after 2 nd blood taking
A	0.93 ± 0.358	1.608 ± 0.423	+ 73.0%	1.562 ± 0.482	+ 68.0%	- 5.0%
B	1.68 ± 0.457	2.554 ± 0.585	+ 52.0%	2.486 ± 0.465	+ 48.0%	- 4.0%
A+B	1.358 ± 0.441	2.199 ± 0.575	+ 62.0%	2.140 ± 0.479	+ 57.6%	- 4.4%

Data are means ± standard deviation (SD).
The Student's t test has been used for all samples.

**Figure 3:** Total antioxidant capacity (TAC) in blood serum before (1st blood collection) and after (2nd and 3d blood collection) diet.

Significance, according to Student's t-test. Data are representative of means ± SD

TAC in serum after diet rich in antioxidants was significantly higher (2nd blood collection): $p=0,000$. TAC values remained high one week after the diet (3d blood taking): $p=0.119$.

with nutrition was examined (Table 2, Figure 3). Increased serum antioxidants levels were observed after the 30-days diet rich in antioxidants in 70% of the volunteers ($p=0.000$). The increase reaches in some subjects 270%. Thirty per cent of the subjects who did not present any increase, did not follow the diet as gathered by the dietary records they kept. After the third blood collection, there was a low not significant decrease in serum antioxidants ($p=0.119$).

Discussion

Oxidative stress and age

The present study analyzed the antioxidant capacity of three different age groups. As a primary result, the mid-

dle-aged and elderly subjects showed higher antioxidant capacity than the younger subjects. Previous studies have acknowledged age-related increase of oxidative stress^{25,27}.

The results suggested that oxidative stress in young people is independent from their current stress (most of them were students and participated in the survey some days before their semester exams) and may be explained with their diet and daily routine (Figure 2). On the other hand, especially in Greece, elderly people have a better diet than the younger ones. The oxidative stress might increase with malnutrition and stress⁴⁵ and is not so relevant with age. Previous studies have reported blood GSSG/GSH increase with age, suggesting that glutathione redox status inclines to a direction of the oxidation with age^{50,51}. Another study, however, displayed constant balance in blood GSSG/GSH despite increase of plasma oxidative stress with age⁵². Another major serum component, which could be responsible for the increased TAC in elderly people, is urate. For example, TAC is increased in hemodialysis patients, despite the increased oxidative stress that characterizes them and this is due to increased urate levels.⁵³ Our study presumed these findings. Although the cause of this hypothesis is not clear, it is assumed that good health and eating habits of these middle-aged and elderly subjects may be factors in well-balanced antioxidant capacity.

Young people and especially students are under biological and emotional stress. This may lead to high cortisol levels, which can be an additional cause for antioxidants reduction. From our results, we assume that stress is not the reason of lower antioxidant levels in young people.

Oxidative stress and nutrition

Our findings highlight the possibility that antioxidant activity in the organism could be increased by high dietary intakes of fruit, vegetables and other rich in antioxidants constituents^{23,24,36}. A positive, statistically significant correlation with antioxidant rich diet was observed. The volunteers, who did not follow the diet, did not present

any increase in serum TAC. Differences in each individual metabolism, daily activities^{36,38} and needs, as well as their preference in food combinations may explain the differences observed in diet response. The bioability and metabolism of food antioxidants are different and influence antioxidant response^{54,55}. Vitamin E, for example, can act under special conditions as a prooxidant and not as an antioxidant. Such a condition takes place *in vitro* in the presence of sufficient amounts of vitamin E with concomitant depletion of other antioxidants that are necessary for vitamin E reduction, like ascorbate. This results in accumulation of the oxidized form of vitamin E that is able to oxidize lipids^{56,57}. Besides certain food combinations may counteract the absorption and protective effects of flavonoids. Moreover, the diet may also contain prooxidants such as iron, copper and lipid peroxides^{58,59}.

In conclusion, the significance of a balanced rich in antioxidants diet instead of single antioxidants administration is the major finding of the present study. There is also a need to take into account and more precisely study dietary combinations and habits to confirm these results.

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