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Is antioxidant plasma status in humans a consequence of the antioxidant food content influence?

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Abstract. – ***Background:*** **A constantly growing part of consumers considers the organic foods healthier than the conventional foods. However, so far few nutritional intervention studies in humans are available on the comparison of organic and conventional food products.**

Objective: **The aim of the present work was to compare the total antioxidant activity of organic versus conventional fruits (apples, pears, red oranges, lemons, strawberries and bananas), vegetables (lettuces, tomatoes, onions, garlics, carrots, beans, potatoes, celeries, peas, courgettes and zucchinies), red wine and milk.**

Design: **The oxygen radical absorbing capacity (ORAC) procedure was used to determine both the antioxidant activity of food from organic or conventional origin, and the human plasma antioxidant capacity. Ten Caucasian Italian men, aged 30-65 years, were recruited. The subjects were healthy, according to the clinical examination and the disease history, none smoked or took any drug. Anthropometric parameters of all the participants were measured.**

Results: **The ORAC values of most part of organic foods, i.e. fruits, vegetables as well as red wine and milk were significantly ($p < 0.005$) higher than those of the conventional homologues. Three organic foods, i.e. pears (–25%) ($p < 0.01$), lettuces (–20%) ($p < 0.01$), and tomatoes salsas (–4%) showed ORAC values lower than the conventional homologues. After the consumption of 14 days Mediterranean organic diet a significant ($p < 0.005$) increase (21%) of the human plasma total antioxidant capacity was observed.**

Conclusions: **Our results clearly show that the organic food products have an higher total antioxidant activity and bioactivity than the conventional foods. The results could be used in public health campaign to increase the consumption of products able to provide a significant health protection and prevention of chronic diseases.**

Key Words:

Antioxidant status, ORAC, Organic and conventional agriculture.

Introduction

Several observational studies and clinical trials have provided scientific evidence that diets rich in fruits, vegetables, legumes, whole grains, fish, and low-fat dairy products are associated with lower incidence of various chronic diseases[1,2]. Many scientific papers have focused on the relationship between free-radical-induced oxidative stress and the progressive increase in the risk of disease[3-5]. Fruits and vegetables contain antioxidant compounds broadly called polyphenols that are known to reduce oxidative stress and prevent chronic diseases[6-11]. The antioxidant properties of these compounds are responsible for their anticancer, antiviral and anti-inflammatory properties[12]. They can also prevent capillary fragility and platelet aggregation[13,14].

To encourage a vegetable consumption through nutritional counselling, it is important to

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bear in mind that not all vegetables have the same phenolic composition and not all phenolics have the same antioxidant capacity[15-17]. It is therefore important to recognize which vegetables have the highest antioxidant capacity and introduce them regularly into the diet[18].

As consumers are aware of their health and more and more conscious of environmental conditions, there is an increasing demand for food obtained from alternative cultural practices limiting the use of mineral soluble fertilizers and synthetic pesticides.

According to European Community Regulations (2092/91/ECC and updatings), "organic" plant foods are those produced without the use of synthetic chemical pesticides and largely without the addition of readily soluble mineral fertilizers. It is thought that in the absence of pesticides, the plants could contain higher levels of antioxidant components as a result of enhanced synthesis of active phytochemicals produced in defence against biotic and abiotic stress[19,20]. It has been suggested[21] that organic product could contain 10-50% more phytochemicals than non-organic product. Although organic product is often compared with product from cultivations based on the use of synthetic fertilizers and pesticides, within the European Community countries these so-called "conventional" methods have been officially replaced by "integrated" systems that reduce the use of chemicals by combining the organic and conventional techniques[22].

To date, there has been few studies on the comparison of organic and conventional food products in nutritional intervention studies in humans.

The aims of the present work were: (a) to compare by the oxygen radical absorbing capacity (ORAC) the antioxidant properties of organic versus conventional foods; (b) to verify if the consumption of organic or conventional foods affects the human plasma antioxidant capacity measured by ORAC.

Subjects and Methods

Study Population Subjects

Ten Caucasian Italian men, aged 30-65 years, were recruited. All them were free from hypertension, cardiovascular diseases and alcohol abuse, none smoked or took any other drug. None was taking oral medication, during the month preceding the beginning of the study or during the study period. The subjects were healthy, according to clinical examination and disease history.

Usual dietary intakes during the previous 12 months were estimated by a semiquantitative food-frequency questionnaire. The alimentary diary was analysed for nutrient composition using diet analyser software INDALI.

All selected subjects followed for first 14 days an adequate nutritional diet using only foods by a conventional agriculture techniques (T0) and for other 14 days organic products (T1). Successively, for other 14 days (T1), these subjects consumed an exclusively organic diet, based on the same prescriptions used in T0; these prescriptions are the same for all studied subjects.

We had the guarantee that all studied subjects had an homogeneous diet and lifestyle because they are enlisted from the same community with a refectory service. No change of total energy intake (kcal/day) was done during the experimental time. No change in Resting Metabolic Rate (RMR) was observed, and the physical activity of the subjects was not different during the time course of the experiment.

All the subjects provided consent to take part to the study according to the guidelines of the "Tor Vergata" University Medical Ethical Committee, Rome, Italy.

Food Sampling and Extraction

All vegetables and fruits were obtained fresh from a local greengrocer in the spring and summer of 2005-2006. Naturally occurring herbs were collected in the spring and early summer of 2006, whereas spices were obtained from a local herb supplier.

In some cases, more than one cultivar was tested.

For each food product, at least four samples were tested, each in duplicate. The following fresh vegetables were analysed: lettuces (*Lactuga romana*, var. Romana); tomatoes (*Solanum lycopersicum L.* var. Miroo a grappolo); onions (*Allium cepa* var. Bianca di maggio); garlics (*Allium sativum* var. Bianco); carrots (*Daucus carota* var. Tancar), beans (Faseolus vulgaris var. Borlotti), potatoes (*Solanum tuberosum*), celeries (*Apium graveolens L.* var. dulce), peas (*Pisum sativum*), courgettes (*Cucurbita pepo*, var. verde di Milano). The following fresh fruits were analysed: apples (*Pirus malus*, var. deliciuos), pears (*Pyrus communis L.* var. Williams), red oranges (*Citrus sinensis*, var. Tarocco), lemons (*Citrus limon*), strawberries (*Fragaria vesca*), bananas (Musa sp.).

Red wine of controlled origin (DOC) was produced in the 2005 in the Lazio Region of Italy.

Dressing: extra-virgin olive oil produced in the 2005/2006 season in the Lazio Region of Italy by local producer, was used.

All tested foods were both from conventional and organic agricultural practices.

Food samples were processed as follows: sample of the pooled fresh edible part of each lot of foods were homogenized using an Ultra-Turrax T8 under nitrogen atmosphere (to preserve oxidation) for 5 min. After homogenizing 5 g of samples were extracted twice in 20 ml of water, and centrifuged at 1800 rpm for 10 minutes. The extracted was stored at –20°C always under nitrogen atmosphere until was analyzed.

Oxygen Radical Absorbance Capacity Assay

The ORAC methodology is arguable the most accepted and accurate indicator of antioxidant status, mainly because it is based on measurements of fluorescence rather than absorbance[23]. This increases sensitivity and so permits a much lower molar ratio of antioxidant sample: reagents, thus minimizing the likelihood of cross-reactions between sample and reagents. In addition, the ORAC methodology measures "total radical scavenging ability", since it is unique in that it takes reactions to completion, permitting a calculation of "total area under curve".

The ORAC assay works by the following principle. A sample is added to a free radical generating system, the inhibition of the free radical action is measured and the results calculated are related to the antioxidant capacity of the sample. AAPH is used as the free radical generator and b-PE is used as a target for free radical attack.

Free radicals cause a conformational changes in the protein structure of b-PE leading to fluorescence quenching in a dose and time-dependant manner.

The original method of Cao et al.[24], with a few modifications, was used[25]. The final reaction mixture for the assay (2 ml) was prepared as follows: 1,750 ml of 75 µM phosphate buffer pH 7.0, +0.100 ml of 20 µM Trolox used as standard, or 0.100 ml of sample, or 0.100 ml of buffer alone used as blank; + 0.100 ml of 34 mg/l β-PE was added in each well. The oxidant reaction was started by the addition of 0.050 ml AAPH 160 mM to each well. The quenching of PE was measured using a Varian Cary Eclipse Fluorescence Spectrofotometer at λ = 546 nm (λ exicitation) and λ = 573 nm (λ emission) and it was monitored every 2.5 min at 37°C for 1 hour or until the fluorescence's variation was less than 2%.

The ORAC value is calculated according to the formula:

$$\text{ORAC (Micromol Trolox Equivalents/g)} = [(As - Ab)/(At - Ab)]\ kah$$

where As is the area under the curve (AUC) of β-PE in the sample, calculated with the Origin 2·8 integrating program (Microcal Software). At is the AUC of the Trolox, Ab is the AUC of the control, k is the dilution factor, a is the concentration of the Trolox in mmol/l, and h is the ratio between the litres of extract and the grams of vegetable or oil used for the extraction.

Chemicals

AAPH (2,2'-Azobis(2-aminopropane)dihydrochloride) was purchased from Polyscience (Warrington, PA, USA). A working solution of 160 mM was prepared fresh by adding 5 ml phosphate buffer to 217 mg AAPH and was stored on ice until used for analyses.

Trolox (6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid) A stock solution (100 microM) was prepared by dissolving 5,0 mg trolox in 200 ml of phosphate buffer. This was further diluted 1:5 v/v to give a working solution of 20 microM.

β-phycoerythrin (β-PE; Sigma-Aldrich) A stock solution was prepared by dissolving 1 ml of PE in 14,7 ml of phosphate buffer. This as diluted further 1:2 to give a working solution.

Statistical Analysis

Statistical data analysis was performed using SPSS statistical package (SPSS Chicago, IL). Descriptive values are expressed as median ± range. The significance of the differences between median was calculated using Mann Whitney Test.

The minimal level of significance was fixed at $p \leq 0.05$ for all the procedures.

Results

Table I shows the ORAC values of conventional and organic foods.

A significant increase ($p < 0.005$) of the antioxidant capacity of the organic group of foods were

Table I. Antioxidant capacity in conventional and organic produts.

	Conventional		Organic		
	Median	Range	Median	Range	_%
Garlic	2572,5	70	3816,5	52	48**
Orange	900	50	1606	56	79**
Banana	205,9	16	339	38,6	65**
Carrot	116,4	27,2	166,8	58,4	43**
Beans	50,4	21,2	207,6	31,2	312**
Strawberry	846,7	37,2	921,2	41,6	9**
Lettuce	756,3	99,8	608,5	80,8	-20**
Limon	1505	54	1603	48	7**
Apple	454	81,9	610,5	47	34**
Potato	298,8	4,4	423,6	50,8	42**
Tomato Souce	205,2	19,8	213,8	58,8	4
Pear	246,4	132,2	185,3	58,4	-25**
Peas	88,2	41,8	164,8	65	87**
Tomato	280,8	72,6	475,2	98,4	69**
Celery	265,7	119,8	414,9	40,4	56**
Wine	3132	280,2	4725	164	51**
Courgettes	774	148,8	894	60,6	15**
Milk	195,8	78,4	216,6	38,6	11*

*$P < 0.05$; **$P < 0.01$ Mann Whitney Test.

observed in bananas (+65%), apples (+34%), lemons (+7%), strawberries (+9%), carrots (+43%), beans (+312%), potatoes (+42%), celeries (+56%), peas (+87%), zucchinies (+16%), garlics (+48%), red wines (+51%) and milk (+11%), whereas pears (–25%) ($p < 0.005$), lettuces (–20%) ($p < 0.005$) and tomatoes salsa (+4%) showed a lower antioxidant capacity respect to the conventional ones.

Plasma total antioxidant capacity was assessed after a consumption for 14 days of a conventional diet (T0) and after a consumption for 14 days of a organic diet (T1) for all studied subjects.

Table II shows the mean of daily and weekly food consumed by the studied subjects. Table II shows mean total energy, macronutrient and micronutrient values for the menus, provided by

Table II. Mean of daily and weekly food consumed by the studied subjects.

Food	Daily average (g)	Weekly average (g)
Milk and yogurt	313	2190
Cheese	80	561
Extra-virgin olive oil	26,7	187
Bread	174	1218
Pasta or rice	129	905
Pulses	60	416
Vegetables	615	4307
Potatoes	51	358
Fruit	662	4638
Non-alcoholic drinks	43	299
Alcoholic drinks	242	1290
Meat	144	1009
Salami	24	171
Eggs	17	117
Saccharose	8,5	59,5
Cakes	63	443
Pizza and sandwich	71	498
Coffee	36	255

Table III. Nutrients' daily intake relative to the group of study.

Elements	Intake (g)
Total proteins	148,94
Total lipids	95,66
Saturated fatty acids	29,72
Monounsaturated fatty acids	38,08
Polyunsaturated fatty acids	9,24
Free glucose	401,72
Starch	242,49
Soluble glucose	127,86
Saccharose	19,43
Lactose	66,88
Glucose	15,42
Fructose	30,34
Raffinose	0,04
Stachiose	0,56
Maltose	0,79
Total fiber	42,59
Cellulose	17,96
Soluble fiber	3,07
Cholesterol	0,35
Alcohol	25,01
Calcium	1,65449
Iron	$19{,}87 \cdot 10^{-3}$
Copper	$3{,}08 \cdot 10^{-3}$
Sodium	2,36
Potassium	5,23
Phosphorous	2,25
Zinc	$12{,}24 \cdot 10^{-3}$
Thiamin	$1{,}65 \cdot 10^{-3}$
Riboflavin	$2{,}54 \cdot 10^{-3}$
Niacin	$23{,}86 \cdot 10^{-3}$
Retinol	$0{,}27 \cdot 10^{-3}$
Carotene	$6{,}26 \cdot 10^{-3}$
Vitamin C	$0{,}35 \cdot 10^{-3}$
Vitamin E	$18{,}09 \cdot 10^{-3}$
Vitamin B6	$3{,}63 \cdot 10^{-3}$
Folic acid	$0{,}68 \cdot 10^{-3}$
Total equivalent in Kcal	3138,8

the analysis of food intakes of all partecipants to the study. When compared to the Recommended Dietary Allowances (RDA), it was found that the meals habitually consumed provided adequate amount for total energy from proteins (23%), carbohydrates (62%), fats (15%) (Figure 1). Micronutrients intakes (vitamins A, D, E, C, B6, B12, thiamin, niacin, riboflavin, folate, calcium, phosphorus, magnesium, iron, zinc and selenium) were according to recommendations. However, percent energy from cholesterol exceeded recommendations, while the amount of polynsatured fatty acid was less of RDA, and the ratio between polynsatured and satured fatty acid was 0.31. Red wine was assumed moderately. The assumption of fruits, vegetables, cereal and olive oil was acceptable. The assumption of meat, milk and milk derivatives exceeded the recommendation.

Baseline fasting plasma ORAC of the study partecipants at T0 and T1 was assessed: antioxidant capacity at T0 was 2.25 mM TE, at T1 was 2.75 mM TE. There was a significant increasing (21%) after the consumption of organic diet.

Discussion

Given the constantly increasing consumers' interest in organic foods, there is a real need to establish on a rigorous scientific basis if organic foods are healthier than conventional ones. Studies comparing foods derived from organic and conventional growing systems were assessed for three key areas: nutritional value, sensory quality, and food safety[26]. As reported by Magkos et al[27], up to now there are only few well-controlled studies that are capable of making a valid comparison. Therefore, a compilation of the results is difficult and a generalisation of the conclusions should be made with caution. For example, there is no evidence that the organic foods may be more susceptible to microbiological contamination than conventional ones. In spite of these limitations, however, some differences can be identified. Although there is little evidence that the organic and conventional foods differ in respect to the concentrations of the various micronutrients (vitamins, minerals and trace elements), there seems to be a slight trend towards higher ascorbic acid content in the organically grown leafy vegetables and potatoes. There is also a trend towards a lower protein concentration but of higher quality in some organic vegetables and cereal crops. It is likely that the organically grown foods are free of pesticides residues, but, as a conse-

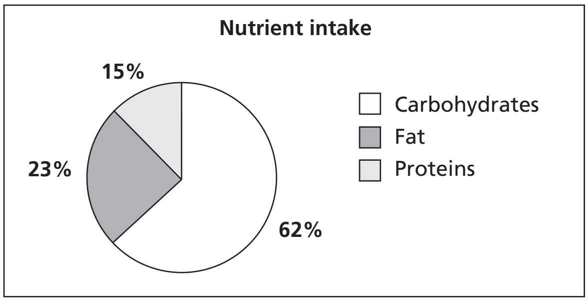

Figure 1. Nutrient intake.

quence, it is also possible that an higher mycotoxins' contamination could occur in these foods[28]. Studies are currently in course in our laboratory to verify it. In this paper we first focused our attention on a single parameter, the total antioxidant activity by ORAC, of evaluation for comparison of the two agricultural systems, integrated or "conventional" and organic or "bio". Veyrat et al.[29] showed that when expressed as fresh matter, the organic tomatoes had higher vitamin C, carotenoids, and polyphenol contents than conventional tomatoes. In this study, we observed a statistically significant differences ($p < 0.005$) between the organic and conventional tomatoes exclusively on the fresh product, but not on transformed salsa tomatoes. We observed that the organic red orange (var. Tarocco) had a total antioxidant activity higher than conventional ones ($p < 0.05$). Consistently with our data Tarozzi et al.[30] reported that the red oranges (var. Tarocco) have a higher phytochemical content (i.e., phenolics, anthocyanins and ascorbic acid), total antioxidant activity and bioactivity than integrated ones. As highlighted in a systematic review[31], the organic foods contain 10-50% more phytochemicals than non-organic ones. The increased synthesis of the phytochemicals in the plants from organic cultivation has been explained as a physiological protective response to the higher exposure to biotic and abiotic stress. Although we did not measure the content of the phytochemicals it is plausible that the higher antioxidant capacity observed for most part of the tested foods is to be related to the phytochemicals' content.

The health-related properties of the phenolic compounds contained in the fruit and the vegetables largely derive from their antioxidant activity. Recent data by Ninfali et al.[32] illustrated the importance of consumption of the vegetables, that are the most important source of phenolic in the Mediterranean diet, to provide a significant protection against the chronic diseases.

Conscious that, regardless of its organic or conventional origin, a well-balanced diet is necessary to improve the health and that the administration of a single or few organic foods would not evidence any possible beneficial effects, we decided to conduct our study by comparing the effectiveness of a Mediterranean diet based on conventional versus organic foods in modulating the antioxidant plasma status.

Our data highlight a possible impact on human health of a Mediterranean diet comprising the organic products versus conventional, due to the effect on the total plasma antioxidant capacity. In particular, an increase in the plasma antioxidant capacity was observed in the subjects receiving the organic diet.

As the central obesity correlate closely with hyperinsulinemia and insulin resistance, and with the possibility of developing the type 2 diabetes and the coronary heart diseases, both in obese and in metabolically obese individuals[33,34], the weight management can help to reduce the number of people at risk for the diabetes and the risk of the cardiovascular disease (CVD) complications or the premature mortality[35,36]. Moreover, the supplementation of natural antioxidants through a balanced Mediterranean diet particularly rich in fresh organic products could be an effective and also economic way to increase a resistance of biomolecules to the oxidative stress in populations with habitually low intakes of the antioxidant micronutrients.

Several important directions can be highlighted for future research; we found that the fasting baseline plasma ORAC of the participants of the study was correlated with their daily estimated intake of the total antioxidant from organic foods. These results could be used in public health campaign with the aim to increase the consumption of products able to provide a significant health protection and prevention of the chronic diseases.

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