HEALTH PROMOTING EFFECT OF FRUIT AND VEGETABLES CONSUMPTION: MECHANISM OF ACTION IN HUMANS

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Summary

In vivo O2 utilization for catabolic processes is associated with the generation of Reactive Oxygen Species (ROS). Excessive production of ROS can cause Oxidative Stress, which is the unbalance between ROS and antioxidant defenses. ROS, by causing oxidative damage to biological macromolecules such as DNA, lipids, carbohydrates and proteins, are believed to be involved in the initiation and progression of several chronic diseases, including coronary heart disease (CHD) and cancer. Oxidative stress can be modulated by the diet, epidemiological research has shown an inverse relationship between fruit and vegetable consumption and the incidence of degenerative diseases. The most plausible explanation for this association is that foods of plant origin, through their high content in antioxidant compounds, could reduce oxidative stress and, therefore, prevent oxidative damage to macromolecules. Antioxidant supplementation trials, although not conclusive on this regard, have leading to contrasting results, highlighting the fact that other compounds have to play a role in the health effects of plant food. The scientific community has recently put new hopes in the preceding hypothesis by identifying a class of compounds widely present in plant foods endowed with a strong antioxidant activity: the phenolic compounds. This paper aims to summarize the health-promoting benefits of fruit and vegetable consumption with special focus on the most recently suggested role played by phenolic compounds in decreasing the risk of degenerative diseases.

Riassunto

L’utilizzo dell’ O2 durante i processi catabolici utilizzati dalle cellule per produrre energia, portano alla formazione delle Specie Reattive dell’Ossigeno (ROS). L’eccessiva produzione di tali molecole reattive può causare stress ossidativo, un’alterazione del bilancio tra ROS e difese antiossidanti. Lo stress ossidativo indotto dai ROS può causare un danno ossidativo alle macromolecole biologiche come DNA, lipidi, carboidrati e proteine. Tale danno sembrerebbe essere coinvolto nell’iniziazione e nello sviluppo di alcune patologie cronico degenerative come aterosclerosi e cancro.
Lo stress ossidativo può essere modulato dalla dieta e una larga mole di evidenze epidemiologiche ha mostrato una correlazione inversa tra consumo di frutta e verdura e incidenza di disturbi degenerativi. La spiegazione più accreditata per spiegare tale effetto è legata all’elevato contenuto in mole-
cole antiossidanti dei cibi di origine vegetale, antiossidanti che, impedendo l’insorgere dello stress ossidativo prevengono il danno alle macromolecole e di conseguenza riducono il rischio d’insorgenza delle patologie da stress ossidativo. Studi di supplementazione con singoli antiossidanti galenici hanno fornito risultati contraddittori, suggerendo che altri composti sono legati all’effetto protettivo dei cibi di origine vegetale. Recentemente la comunità scientifica ha riposto nuovo speranza nell’identificazione di una classe di composti presenti nei cibi di origine vegetale e dotati di una potente attività antiossidante: i composti fenolici.
Questo lavoro si prefigge lo scopo di descrivere gli effetti benefici sulla salute prodotti dal consumo di frutta e verdura con particolare attenzione alle recenti evidenze sperimentali che suggeriscono un ruolo attivo svolto dai composti fenolici nella diminuzione del rischio d’insorgenza delle patologie degenerative.
INTRODUCTION

Reactive Oxygen Species (ROS) cause oxidative damage to biological macromolecules such as DNA, lipids, carbohydrates and proteins (1), alter gene regulation, and impair immune function, altogether impairing normal body functions. At a macromolecular level, ROS are known to be involved in the development of numerous physiologic and pathologic degenerative processes, including aging, CHD, some forms of cancer, rheumatoid arthritis, etc. (1, 2). To counteract the oxidative stress lead by ROS, the human body is equipped with a battery of enzymatic (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic (vitamin E, vitamin C, carotenoids and phenolic compounds) antioxidant defenses.

The human diet contains an array of compounds with antioxidant activity able to avoid oxidative damage. The link between diet and disease might be seen, thus, as the result of the imbalance between oxidants production and antioxidant intake, as supported by the large body of epidemiological evidence showing a strong inverse correlation between consumption of food rich in natural antioxidants such as fruit and vegetable and risk of degenerative disease (3, 4).

The following paper intends to summarize the health-promoting, chronic disease-preventing benefits of diets rich in plant foods, and especially those that could be related to their high content in phenolic compounds as the main source of antioxidant activity.

DIET, OXIDATIVE STRESS AND DISEASE

Since the concept of Mediterranean diet emerged in 1960 (5), 20 years of biochemical, clinical and epidemiological research have provided solid foundation for its attributed health benefits. Even though the definition of Mediterranean diet is neither straightforward nor exact, the Mediterranean diet was described in the early 1960s as follows (6): abundance of plant foods minimally processed, seasonally fresh, and locally grown; olive oil as the principal source of fat; moderate amounts of dairy products daily; low to moderate amounts of fish and poultry; scarcity of red meat, and moderate wine intake. This diet, low in saturated fat (<7-8% of energy), was the cornerstone of a whole lifestyle, which included also regular physical activity. Nowadays, despite the many changes taking place in the dietary style of Mediterranean countries, the moderate consumption of wine, milk and dairy products, low consumption of meat and meat products, high monounsaturated/saturated fat ratio, and high consumption of fruit and vegetables does still apply to the so called “Mediterranean diet”.

The popularity of Mediterranean diet is largely due to the work of Ancel Keys who, in the 60’s, conducted an investigation in seven countries (Greece, Italy, Yugoslavia, Japan, Finland, Netherlands and USA) involving 12763 men followed up over a period of 30 years (5). Dietary data was examined in relation to biochemical variables. The results showed that Northern European populations had an increased risk of CHD compared to Southern Europeans and that saturated fat intake could partially account for the reported difference in incidence.

In the 80’s it became apparent that our understanding of the pathogenesis of CHD was incomplete. Experimental evidence showed that the lipid hypothesis was incapable of fully accounting for the disease. Subsequent research has suggested that other factors may also be involved in the health-promoting action of the Mediterranean diet, especially its antioxidant content.

Epidemiological studies overwhelmingly agree in the protective effect of fruit and vegetables against degenerative diseases such as cancer or
CHD (3, 7). A number of mechanistic hypotheses invoking specific components of these foods have been proposed to explain the findings. One of them, the so called “antioxidant hypothesis”, postulates that health maintenance requires antioxidant protection of cellular compartments against the oxidative damage caused by ROS (8). When oxidative stress overcomes plasma antioxidant capacity, structural components are modified through their interaction with ROS and lose their original role within the cell machinery. The result is the development of the disease. For example, it has been demonstrated that, in addition at high cholesterol levels in plasma (classical cholesterol hypothesis), lipid oxidation is mandatory for the early atherosclerotic lesion to appear (9). Similarly, oxidative damage to proteins and nucleic acids has been identified as a mechanism responsible for the initiation of the neoplastic chain (1). Diets rich in fruit and vegetables, which contain powerful antioxidant compounds, could play a significant role in reducing the incidence of such diseases by preventing macromolecules malfunction through oxidation.

On the basis of the epidemiological studies and supported by the antioxidant hypothesis, numerous small- and large- scale intervention trials have examined the efficacy of antioxidant nutrients in disease prevention with contrasting results. In a prospective trial on vitamin E supplementation and risk of CHD conducted on 87000 healthy female nurses 34 to 59 years of age, Stampfer and colleagues (10) found a 40% reduction in the incidence of CHD over an 8-y period in the supplemented group compared to placebo. Interestingly enough, another randomized (11), double blind, placebo controlled trial on 22000 male physicians treated with 50 mg of β-carotene on alternate days for 12 years did not evidence any statistically significant benefit from β-carotene supplementation regarding the number of deaths from CHD, cancer, or all causes. To complete the spectrum, (12) a large multi-center, randomized, double-blind placebo controlled trial on the efficacy of daily supplementation with beta carotene + vitamin A (30 mg and 25000 UI, respectively) in lung cancer prevention among smokers, the RR of developing lung cancer was of 1.28 for the supplemented group compared to placebo. Moreover, mortality rate from all causes was 17% higher in the treatment group. The examples above suggest that specific antioxidant supplementation does not universally protect against CHD and cancer as fruits and vegetables do. Antioxidant type, combinations, doses, and the characteristics of the population considered are variables to account for before widely recommending antioxidant supplementation to prevent degenerative diseases. At this point, it became clear that supplementation with antioxidant vitamins alone could not fully account for the protective effect of plant food and additional studies were needed to further clarify the causative agents of the health benefits inherent to plant foods consumption.

In 1993, Frankel and colleagues (13) showed that the phenolic compounds present in red wine inhibited human LDL oxidation better than vitamin E did. They hypothesized that phenolics, thanks to their antioxidant properties, might explain the so called “French Paradox”, that is, the apparent compatibility of a diet rich in saturated fat and a low incidence of CHD in some regions of the France. Suddenly the attention of the scientific world turned to these little-studied substances that revealed to be one of the most powerful natural antioxidants present in the diet. Phenolics are a large family of more than 4000 natural compounds widely distributed in plant foods (onions, cabbage, strawberry, grape, blackcurrant, apple etc.) and beverages (wine, tea, cider, beer, cocoa etc). Structurally, phenolics are characterized by the presence of one or more benzene rings bonded to different chemi-
cal groups. They are classified in three classes: simple phenols and phenolic acids, hydroxycinnamic acid derivatives, and flavonoids. Flavonoids are the largest class of phenolics, including a variety of subgroups: flavonols, flavones, flavanones, catechins, isoflavones, and anthocyanidins.

The first epidemiological study correlating flavonoid intake with a reduction in the mortality rate by CHD was published in 1993 by Hertog and colleagues (14). Thereafter, other studies showed a significant inverse correlation between flavonoid intake and total and coronary mortality (15), stroke incidence (16) and cancer (17).

The role that phenolics are thought to have in the prevention of oxidative stress-linked diseases is associated mainly with their antioxidant ability, which has been well established in vitro. Phenolics have been found to possess antioxidant and free radical scavenging properties (18) and to be able to inhibit LDL oxidation (18). They can also inhibit several key enzymes in cellular systems involved in the generation of ROS, including cyclooxygenase, lypoxygenase and NO synthase (19, 20).

The main concern regarding phenolics efficacy was that, until few years ago, there was no prove of their effects in vivo in the human being. We studied the impact of ingestion of phenolic-rich foods on plasma Total Radical-trapping Antioxidant Parameter (TRAP, which is an in vivo marker of plasma total antioxidant capacity) and on phenolics plasma levels in two different studies. In the first study 10 healthy volunteers drunk, fasting in the morning, 300 mL of water, green and black tea, alcohol-free red and white wine (21-23). When subjects drunk green tea, TRAP values rose at 30 (P < 0.05) and at 50 min (P < 0.01). After Black tea ingestion, the TRAP peak was at 80 min (P < 0.01). Red wine raises the plasma antioxidant capacity after 30 min (P < 0.05) with a peak at 50 min (P < 0.01), falling back to baseline values 2 hours post-ingestion. In the white wine and control groups there were no changes in plasma TRAP values within the 2hrs following its consumption. The increase in plasma antioxidant capacity after red wine and green tea ingestion was parallel and highly correlated to the increase in total and single phenolic plasma levels.

The second study, we determined whether human plasma antioxidant capacity (TRAP) responded to the acute ingestion of fresh lettuce identifying the bioactive antioxidant molecules there involved. The study (cross-over design) was conducted on eleven healthy volunteers divided in two groups (A and B), blood samples were collected before and 2, 3 and 6 h after the ingestion of 250 g of fresh lettuce (group A) and without lettuce (group B) (phase 1). The protocol has been repeated after three days (phase 2) on both groups, switching the treatments (group A without lettuce and group B with 250 g. of lettuce). Fresh lettuce consumption significantly raises TRAP at 2 hrs (P < 0.02), peaking at 3 hrs (P < 0.003) and start to decrease after 6 hrs. In the control group, there were no changes in plasma TRAP for all over the period of observation. Cumaric acid and quercetin plasma levels significantly increased at 2 hours (P < 0.004) and was close to significance (P < 0.06) for caffeic acid. Quercetin (P < 0.002), caffeic acid (P < 0.04) and cumaric acid (P < 0.003) concentrations reached the peak after three hours. Plasma carotenoid concentrations was not modified by the ingestion of fresh lettuce, except for _-carotene at T6 (P < 0.05). On the contrary, vitamin C concentration was significantly raised at 2 hrs (P < 0.001), 3 hrs (P < 0.0008) and 6 hrs (P < 0.0003) by the ingestion of fresh lettuce. In the control group there was no changes in individual antioxidant plasma levels for all the period of observation. Ingestion of fresh lettuce increased plasma antioxidant capacity in healthy subjects through a change of vitamin C, caffeic
acid, cumaric acid and quercetin plasma levels. Our results suggest that ingestion of plant foods and products provide a vast array of bioactive compounds able to modulate red-ox status.

In order to clarify the mechanism of action of phenolics, we conducted experiments on 6 phenolics to establish which one was the more efficient scavenger of different ROS and which were their effects on LDL oxidation. The selected phenolics of different chemical structure were: Quercetin (Q), Catechin (C), Rutin (R), Gallic Acid (GA) and Caffeic Acid (CA). The results were compared to the hydrosoluble form of vitamin E: Trolox (T). We observed that Q was the most efficient scavenger of the peroxyl radical, followed by C > R > CA > GA = T. Quercetin also showed the best antioxidant efficiency in scavenging the hydroxyl radical: Q > R > T > CA > C > GA. Quercetin was the most efficient in protecting LDL from oxidation driven by peroxyl radicals followed by T > R > C > CA = CG. Moreover, when LDL were oxidized with ferrylmyoglobin, Q displayed the strongest protective effect: Q > T > R > C > CA = GA. In summary, our results indicate that the selected phenolics are efficient antioxidants in scavenging ROS. They protect human LDL from oxidative damage and exhibit an antioxidant behavior with physiological relevance. Quercetin is, among the tested compounds, the most efficient antioxidant.

**CONCLUSIONS**

The existing scientific evidence regarding a primary role for fruit and vegetables in preventing degenerative diseases is consistent and trustworthy. Thus, an increased consumption of plant food can be recommended. However, even though the protective effect of plant food seems to be mediated by its high content in natural antioxidants, data on antioxidant supplementation are contradictory and needs of further investigation.

The demonstration that phenolics are bio-active dietary compounds able of raising plasma antioxidant defenses in man has far-reaching implications and shed further light to the nature of the beneficial effects of plant food consumption. Although more and better information on their bioavailability and in vivo efficacy is needed, phenolics seem to be a class of compounds to investigate in the attempt to clarify the association between diet, oxidative stress and disease. However, it is becoming clear that the protective effect of plant food cannot be explained only on the basis of their content in single antioxidant molecules, but it is the overall and variegated composition of the food item that is at the basis of the protective effect.

In summary, the habitual “overloading” of vitamin supplements, common practice in Western countries, should be curbed until conclusive evidence on the safety of long-term antioxidant supplementation is available. In healthy subjects, the recommended optimal intake of antioxidants should be preferentially achieved by increasing plant food consumption through a well-balanced diet rather than in “galenic” preparation.
References


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