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Review

# The Antioxidative Protecting Role of the Mediterranean Diet

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**A B S T R A C T**

*Recent meta-analysis shows that adherence to a Mediterranean diet (MD) can significantly decrease the risk of overall mortality, mortality from cardiovascular diseases, as well as incidence of mortality from cancer, and incidence of Parkinson's and Alzheimer's disease. All of these diseases could be linked to oxidative stress (OS) as antioxidative effect of MD is getting more attention nowadays. Although a lot of research has been done in this area and it suggests antioxidative protective role of MD, the presented evidence is still inconclusive. The aim of this paper is to review studies investigating the effect of MD on OS, as well as to identify the areas for further research.*

***Key words**: Mediterranean diet (MD), oxidative stress (OS), antioxidative property, oxidative stress biomarkers*

## Introduction

For decades, researchers have been intrigued by the apparent health benefits of the Mediterranean diet (MD). A lot of research has been done since the first classical study in 60's[1–3] and there is numerous evidence of MD's beneficial effects. However, there is not a plausible explanation linking MD to all of the collected evidence. Different mechanisms of action were proposed for the beneficial effects of MD, some of the most commonly reported being antiinflamatory, antithrombotic, as well as, antioxidative. Here are some of the clinical findings that support proposed mechanisms of the MD effects: the change of carotid intima-media thickness[4]; down-regulation of cellular and circulating inflammatory biomarkers related to atherogenesis (serum C-reactive protein, interleukin-6 (IL6) and endothelial and monocytary adhesion molecules and chemokines) in subjects at high cardiovascular risk[5]; and favorable effects of MD on plasma lipid profile (reduction of total and plasma LDL cholesterol levels, plasma triglyceride levels, and apo-B and VLDL concentrations, and an increase in plasma HDL cholesterol levels)[6].

For a long time the focus of research in MD was on olive oil as the main source of fat in MD. Therefore, the benefits on cardiovascular system, known from epidemiological studies were explained through the effect of MD on lipid profile. Recent meta-analysis shows that adherence to a MD can significantly decrease the risk of overall mortality, mortality from cardiovascular diseases, as well as incidence of mortality from cancer, and incidence of Parkinson's and Alzheimer's disease[7]. There is also large number of evidence showing that MD may have protective role against obesity and is associated with reduced risk of metabolic syndrome[8]. All of the mentioned evidence could not be linked only to the influence on lipid markers. However, all of the above mentioned diseases where MD has been proofed as beneficial could be linked to the oxidative stress (OS). Therefore, anti-oxidative effect of MD is getting higher attention nowadays. Although great success has been made in the accumulation of solid scientific background underlying this topic, there are still a lot of controversies and full mechanisms of protection is far from being established. This is partly because measurement of OS is usually based on indirect and nonspecific measurement of products of reactive oxygen species (ROS), and there are no standardized or routine methods of measuring OS. Moreover, measuring

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the anti-oxidative effect of particular diet is an even bigger challenge. Numerous parameters aggravate such a measurement, some of them being: different nutrients of the diet, the effect of physical activity, lifestyle and many environmental factors, which all influence the OS. The aim of this paper is to review studies investigating this problem, as well as to identify the areas for further research. For that purpose, first we reviewed the diseases in which OS has been involved, explaining the known mechanism. Then, we reviewed the individual components of MD, known to have anti-oxidative effect. Furthermore, we present the methods used to measure OS in those studies. At last, we present the evidence of the effect of MD in different patient groups and discussed types of studies done, as well as the need for further investigation. Taking these numerous objectives into consideration, one should keep in mind that limitation of this review is oversimplification of some of the rather complex mechanisms. However, the authors wish to present the big picture of the subject of interest, accounting for this important limitation.

## Oxidative Stress in Pathology of Chronic Diseases

OS, an imbalance between the generation of ROS and antioxidant defense capacity of the body, plays critical roles in the pathogenesis of various diseases. In the diabetic condition, OS impairs glucose uptake in muscle and fat[9,10] and decreases insulin secretion from pancreatic β cells[11]. Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins[12]. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. These consequences of OS can promote the development of diabetes mellitus complications[13].

Increased OS also underlies the pathophysiology of hypertension[14] and atherosclerosis[15] by directly affecting vascular wall cells. ROS are key mediators of signaling pathways that underlie vascular inflammation in atherogenesis, starting from the initiation of fatty streak development, through lesion progression, to ultimate plaque rupture. Many data support the notion that ROS released from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, myeloperoxidase, xanthine oxidase, lipoxygenase, nitric oxide synthase and enhanced ROS production from dysfunctional mitochondrial respiratory chain, indeed, have a cause-effect role in atherosclerosis and other vascular diseases[16].

Evidence also supports the notion that obesity *per* se may induce systemic OS and that increased OS in accumulated fat is, at least in part, the underlying cause of dysregulation of adipokines and development of metabolic syndrome[17]. Like in other conditions (i.e. diabetes, atherosclerosis, hypertension), OS works here in conjunction with inflammation. Evidence of inflammatory processes in accumulated fat also appears to be an early initiator of metabolic syndrome. Likewise, the more active angiotensin system in obesity may contribute to even greater OS that serves as a key signaling event in vascular remodeling. These factors strengthen obesity's association with OS.

Growing data from experimental models and human brain studies suggest OS may play an important role in neuronal degeneration in diseases such as Alzheimers' and Parkinson[18]. It is still unclear whether OS is the primary initiating event that is associated with neurodegeneration in Parkinson and Alzheimers' disease. However, a growing body of evidence implicates it as being involved in at least the propagation of cellular injury that leads to neuropathology in these various conditions. It is intimately linked with an integrated series of cellular phenomena, which all seem to contribute to neuronal demise. Interaction between these various components is not necessarily a cascade but might be a cycle of events, of which OS is a major component[19].

Furthermore, increased formation of ROS can promote the development of malignancy, and the žnormal' rates of ROS generation may account for the increased risk of cancer development in the aged. Indeed, knockout of various antioxidant defense enzymes raises oxidative damage levels and promotes age-related cancer development in animals[20].

Although, OS has long been linked to the pathology of the above mentioned diseases, the whole mechanism is still unclear, leaving an open question whether it is a primary cause or merely a downstream consequence of those conditions.

## Mediterranean Diet and its Components

There are many variations to the MD, due to social, political and economic differences between countries in the Mediterranean region. The concept of the MD is based on dietary habits more typical of the 1960s, and rather different for the most part to present Mediterranean lifestyles.

The traditional MD is characterized by a high intake of vegetables, legumes, fruits and nuts, and cereals (that in the past were largely unrefined), and a high intake of olive oil but a low intake of saturated lipids, a moderately high intake of fish (depending on the proximity of the sea), a low-to-moderate intake of dairy products (and then mostly in the form of cheese or yogurt), a low intake of meat and poultry, and a regular but moderate intake of ethanol, primarily in the form of wine and generally during meals[21].

In the past, major emphasis was put on the low saturated fat content of the MD while more recent evidence has underlined the importance of plant foods rich in antioxidants and omega-3 fatty acids. The Lyon Diet Heart Study, which was the first clinical trial to show evidence of health benefits of the MD, proposed that beneficial components of the MD could be antioxidant-rich foods

including vegetables, fruits, and their derivatives such as vinegar, and omega-3-rich fish and canola oils- not olive oil[22]. Many studies suggest that a link exists between fruit and vegetables in the diet or the amounts of plasma antioxidant vitamins (ascorbic acid, tocopherol and carotenoids) and a risk of death from cancer or coronary heart diseases[23].

*Fruits and vegetables*

Public health campaigns recommend increased fruit and vegetable consumption as an effective mean of cardiovascular risk reduction. Regular fruit and green vegetable consumption leads to the accumulation of nitrate / nitrite / nitric oxide, polyunsaturated fatty acids and polyphenol compounds. These compounds play an important role in protection against myocardial infarction[24] and are associated with OS. Fruit and vegetables are rich sources of antioxidants, including vitamins, flavonoids, carotenoids, etc. Water soluble Vitamin C and fat soluble vitamin E both have well established anti-oxidative properties. They enhance immune function, protect the body from free radical damage, and play a role in atherosclerosis. Besides vitamins, carotenoids (such as lycopene, beta-carotene and lutein) are important antioxidants in fruits and vegetables. Lycopene is a red pigment, found in many red fruits and vegetables, although the quantity is higher in cooked tomatoes. Tomato extract or derivative diet supplements have shown to ameliorate hypertension and cardiovascular disease, to protect the skin against the sun (i.e. ultraviolet rays), to decrease the risk of many chronic diseases including cancer and is antioxidant in many processes[25]. Sanchez-Moreno and coworkers[26] reported that the consumption of gazpacho, a typical Spanish tomato-based raw vegetable soup, reduced F2-isoprostanes, a marker of OS, and increased plasma vitamin C in healthy subjects.

Brassica vegetables, including cabbage, kale, broccoli, Brussels sprouts, and cauliflower, have been shown to have protective effects against cancer, probably due to their relatively high content of glucosinolates[27,28]. Glucosinolates exhibit their effect by inducing Phase I and Phase II enzymes, inhibiting the enzyme activation, modifying the steroid hormone metabolism and protecting against oxidative damages[29]. Furthermore, indole-3-carbinol is produced by the breakdown of the glucosinolate glucobrasicin. Indole-3-carbinol is the subject of on-going biomedical research into its possible anti-carcinogenic, anti-oxidant, and anti-atherogenic effects. Some other bioactive compounds with anti-oxidant activity are: organosulfur compounds (allicin) found in garlic, onion and leek; flavonoids (quercitin, kempferol, catechin) in apples, berries and lettuce; as well as isoflavones (genistein, daidzein) in legumes[30]. All of the mentioned substances are characteristic of MD and showed their antioxidant properties. However, it is very hard to distinguish their antioxidant *versus* other protective mechanisms.

*Olive oil*

Olive oil (Olea europaea) consumption was associated with a lower coronary risk and with a reduced breast cancer risk[31,32]. Olive oil is rich in several microcomponents that have antioxidant potential[33]. Oleic acid was shown to prevent in vitro LDL oxidation[34]. Phenolic compounds present in olive oil might also contribute to health benefits derived from the MD.

They reduce the cardiovascular risk factors and down-regulate cellular inflammatory pathways related to atherosclerosis, therefore, it seems appropriate to recommend the intake of olive oil to patients in all stages of atherosclerosis disease[35].

The major phenolic compounds identified and quantified in olive oil belong to three different classes: simple phenols (hydroxytyrosol, tyrosol); secoiridoids (oleuropein, the aglycone of ligstroside, and their respective decarboxylated dialdehyde derivatives); and the lignans [(+)-1-acetoxypinoresinol and pinoresinol][36]. All three classes have potent antioxidant properties. In in vitro[37,38] and animal models[39,40], olive oil phenolic compounds protected lipids from oxidation in a dose-dependent manner. Olive oil's richness in antioxidants may benefit arterial blood pressure, reduce LDL-cholesterol, improve diabetes, and reduce the risk of thrombosis[25].

*Polyunsaturated (PUFA) and monounsaturated (MUFA) fats*

Besides olive oil, the main source of MUFA in MD are nuts, while source of PUFA is fish, as well as nuts. Regular consumption of daily serving of mixed nuts (walnuts, almonds and hazelnuts) has a beneficial effect on some metabolic syndrome features (high waist circumference, hipertriglyceridemia, elevated blood pressure, elevated fasting blood glucose level, low HDL cholesterol level), which demonstrate that regular nut consumption is associated with reduced risk of developing coronary heart disease[41].

Beneficial properties of these components of the diet are related more to anti-inflammatory and vasodilatory than to antioxidative properties[25].

*Alcohol*

In most of the Mediterranean, wine is consumed in moderation and usually taken with meals. Studies have shown that moderate wine consumption increases the antioxidant effects of a MD and decreases oxidative damage[42]. Findings from case control study in southern Croatia, where alcohol consumption is highly prevalent, demonstrated that moderate alcohol consumption was protective factor for acute miocardial infarction (OR 0.63)[43]. Wine, especially red wine, contains a vast array of phytonutrients. Among them, powerful antioxidants polyphenols, protect against LDL oxidation and other pathological sequelae of the OS. The polyphenol resveratrol, present in grapes, protect vascular walls from oxidation, inflammation, platelet aggregation, and thrombus formation. Resveratrol may act on multiple levels, such as cellular signaling, enzymatic pathways, apoptosis, and gene expression[25]. There are also some studies that show that wine consumption increases PUFA, omega-3 levels and improves the omega-3: omega-6 fatty acid ratio[44].

## Biomarkers of Oxidative Stress in Epidemiology and Randomized Studies

Many approaches allow evaluation of OS and antioxidant property of particular intervention. Those numerous methodologies are usually categorized into three main approaches: I) Quantification of radicals; II) Quantification of oxidative damage markers and III) Quantification of antioxidant defending system.

In the first group, the well known technique for the direct detection of radicals is electron spin resonance. This technique is time consuming, requires a complex preparation for the samples, and is hardly applicable in clinical practice due to technical constrains. Therefore, this method has no practical use in epidemiological or randomized studies.

For the direct measurement of ROS concentrations the free oxygen radical test (FORT) technique could be used, which is simpler and faster[45]. FORT is based on the Fenton reaction and measures levels of organic hydroperoxides.

However, OS is generally analyzed by measurement of secondary products (oxidative damage markers). Techniques for quantification of oxidative damage markers are numerous. They are often called fingerprinting methods, because they measure specific end products resulting from the interaction of the ROS with biological macromolecules, such as DNA, proteins and lipids. The appearance of these end products serves as proof of the prior existence of ROS that left their footprints in the cell.

Among lipid oxidation products commonly measured markers are malondialdehyde (MDA), thiobarbituric acid reactive substances (TBARS), and a family of isoprostanes. MDA is one of the terminal aldehyde products of lipid peroxidation, while TBARS mainly includes MDA. MDA and other TBARS condense with two equivalents of thiobarbuturatic acid to give a fluorescent red derivative that can be assayed spectrophotometrically. In the family of isoprostanes, F2-isoprostanes are a series of prostaglandin F2-like compounds produced through the radical-catalyzed lipid peroxidation in vivo, independent of cyclooxygenase. F2-isoprostanes are derived mainly through the arachidonic acid pathway[45]. One of the most abundant F2-isoprostane in humans is 8-epi-PGF2alpha (8-isoprostane). Urinary or circulating concentrations of 8-isoprostane is used as the biomarker for systemic OS in the recent years[46,47]. Other commonly used markers of lipid oxidation are circulating concentrations of ox-LDL and autoantibodies against ox-LDL[48]. Fluorescent oxidation products of lipid peroxidation are the most recent biomarker for OS[49]. This fluorescent assay measures the Schiff base products from interactions of lipid oxidation products with proteins, amino acids, and DNA oxidation products.

Evaluation of protein oxidative damage can be accomplished using the carbonyl assay[50]. Carbonyls are produced from the attack of ROS on amino-acid residues in proteins. Several methods evaluate these carbonyls, including a general estimation of the total carbonyl pool; a specific determination using gel electrophoresis techniques; and detections of peroxides, loss of SH groups, loss of fluorescence (eg, tryptophane), chlorination of proteins, nitration of proteins, and hydroxylation of amino acids.

Furthermore, techniques utilized to detect DNA adducts and base modification are HPLC or GC-MS analysis of 8-OHdG after enzymatic hydrolysis of DNA and assessment of oxidative base damage by the single-cell gel electrophoresis, or comet assay[50]. Other methods exist to determine single and doublestrand breaks[51]. Different oxidized adducts of DNA can be determined. Examples are DNA aldehyde adducts, such as deoxyguanosine--malondialdehyde adducts[52], or the end product of the reaction between DNA and 4-hydroxynonenal, the aldehyde formed following exposure to ROS[50] to generate N2-ethenodeoxyguanosine.

Quantification of antioxidant defending system is used as the third group of methods to indicate OS. Common antioxidant enzymes include superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT).

Besides antioxidative enzymes, the antioxidant levels in different biological specimens are measured as well. Plasma concentrations of vitamin C, alpha-tocopherol, carotenes, carotenoids, and uric acid are used to reflect the anti-oxidative defense. Reduced glutathione (GSH), the smallest intracellular thio (-SH), is a very important antioxidant molecule. In the reduction-oxidation reaction, GSH is converted to oxidized glutathione, glutathione sulfide (GSSG). The ratio of GSH to GSSG and the glutathione redox potential for the GSH-GSSG couple (Eh GSH/GSSG) are measures of OS. A high GSH/GSSG ratio is essential for protection against OS[53]. Both glutathione indices may be preferable to either GSH or GSSG alone as an overall indicator of redox status, because they quantify the dynamic balance between oxidants and antioxidants[54]. The higher the GSH/GSSG ratio or the lower the GSSG, the lower the OS.

One of the approaches most commonly used is the measurement of the total antioxidant activity of a biological site. Depletion of one antioxidant molecule causes changes in the level of overall antioxidant molecules and may be evaluated using a variety of techniques including biochemical, immunohistological, spectroscopical, and electrochemical[55]. One of the biomarker for antioxidant capacity is the susceptibility of LDL to metal-induced oxidation, measured as the lag time[56]. The longer the lag time, the greater is the antioxidant capacity of LDL. Another biomarker for antioxidant capacity is total antioxidant capacity (TAC). Several methods have been used to measure TAC including the oxygen radical absorbance capacity (ORAC) assay[57], the Randox Troloxequivalent antioxidant capacity (Randox-TEAC) assay[58], the ferric reducing ability (FRAP) assay[58], and the detection of reactive oxygen metabolite (d-ROMS)[59].

## Randomized and Epidemiology Trials Exploring the Effect of Mediterranean Diet on Oxidative Stress

Research on the association of the MD with OS is limited with inconsistent results (Table 1). To our knowledge, most of the randomized controlled trials (RCT) involved small sample size (20–51 patients)[60–64], with the exception of PREDIMED study[65,66]. The PREDIMED study showed that MD decreased OS after three months of follow up and enhanced antioxidant defense after three years of follow up, when comparing low fat diet with traditional MD (plus virgin olive oil or nuts). Decreased OS was detected by oxLDL in 372 subjects at high CV risk and by MDA in subsample of 71 patients, but no change was observed when measuring GSH-Px in sample of 372 subjects, while antioxidant defense was measured by TAC levels in 187 subjects at high CV risk.

Two smaller, intermediate-term RCTs also showed positive influence of MD on OS, when comparing MD with a high fat diet in male volunteers after three month ingestion[63], and a low-fat isocaloric diet (2500 kcal for men, 2000 kcal for women) in kidney grafting patients over six month intervention[65], respectively.

On the contrary, three RCTs failed to show the favorable effect of a MD on OS in comparison with a Swedish diet[60,62] and very-low-carbohydrate diet[61]. These were 4-week intervention conducted in healthy subjects[60]; a 3-week intervention performed in patients with rheumatoid arthritis[62]; and a two months intervention in overweight and obese women[61]. In these trials, OS has been measured with various markers, such as plasma antioxidants (vitamin C[63], gamma-tocopherol[62], beta-carotene[62], lycopene[62], and uric acid[62]), plasma lipid peroxidation products (TBARS[64] and MDA[62]), antioxidant enzymes (erythrocyte SOD[65], oxidative DNA damage[63], and urinary F2-isoprostane[60]).

Besides these RCT, the epidemiology study ATTICA[67], which involved 3042 healthy subjects from province of Greece, found positive correlation of MD with total antioxidant capacity and those who adhere more to MD (calculated by med diet scores) had higher TAC levels. Moreover, Dai[68] showed in well controlled study of twins that one unit increment in the Mediterranean diet score was associated with 7% higher GSH/GSSG ratio, meaning

**TABLE 1**
RANDOMIZED AND EPIDEMIOLOGICAL TRIALS EXPLORING THE EFFECT OF MD ON OS

| Author/Year of Publication | Subjects | Design of the study | Diet/ Diet groups | Oxidative stress parameter measured | Results |
|---|---|---|---|---|---|
| Razquin et al.[66], 2009 | 187 subjects at high CV risk | RCT,<br>FU: 3 years | I. control = low fat diet (59 subjects);<br>II. TMD+VOO (65 subjects);<br>III. TMD+ Nuts (65 subjects) | TAC (commercially available colorimetric assay kit) | TAC in both TMD+VOO and TMD+nuts groups. Both changes were statistically significant *vs*. control ($p<0.001$) |
| Fito et al.[65], 2007 | 372 subjects at high CV risk (71 subject randomly selected subsample for MDA measurement) | RCT,<br>FU: 3 months | I. control= low fat diet (121 subjects);<br>II. TMD+VOO (123 subjects);<br>II. TMD+ nuts (128 subjects) | oxLDL (enzyme-linked immunosorbent assay using the mAbE6 antibody), | oxLDL ↓ in TMD+VOO and TMD+nuts groups. Only change in TMD+VOO *vs*. low fat group was significant ($p=0.02$) |
| | | | | GSH-Px by spectrophotometry | No changes were observed |
| | | | | MDA in mononuclear cells by HPLC | Changes paralleled those of oxLDL |
| Dai et al.[68], 2008 | 138 monogygotic and dizygotic twin pairs and 21 unpaired twins | Cross-sectional survey | Diet assessed by FFQ; adherence to MD assessed on the basis of a MD score | GSH/GSSG ratio (higher the ratio = lower the OS) | One-unit in the diet score associated with 7% higher GSH/GSSG ratio ($p=0.03$). |
| Pistavos et al.[67], 2005 (ATTICA study) | 3042 healthy subjects from province of Attica (Greece) | Cross-sectional survey | Diet assessed by FFQ; adherence to MD assessed on the basis of a MD score | TAC (immune-diagnostic assay) | TAC positively correlated with diet score; highest tertile of the diet score had 11% higher TAC levels than lowest tertile ($p<0.01$) |

| | | | | | |
|---|---|---|---|---|---|
| Hagfors et al.[62], 2003 | 51 rheumatoid arthritis patients (10 M, 41 F) | RCT,<br>FU: 3 months | I. MD<br>II. control (participants normal diet) | Plasma levels of retinol, antioxidants (α- i γ-tocopherol, β-carotene, lycopγene, vitamin C and uric acid) and urinary MDA | No changes in urine MDA or plasma levels of antioxidants |
| Leigton et al.[63], 1999 | 42 M | RCT,<br>FU: 3 months | I. MD<br>II. Western diet (high fat diet) | Plasma vitamin C and E, TAC, oxidative DNA damage in blood leukocyte DNA (8-OHdG levels) | The MD TAC (28%). The high fat diet ↓ vitamin C levels, and oxidative DNA damage, significantly. Both diets showed lower OS and higher antioxidative status when wine was added |
| Ambring et al.[60], 2004 | 22 healthy subjects (12 M, 10 F) | R-crossover-CT,<br>FU: 4 weeks + 4 weeks wash out + 4 weeks | I. Swedish diet<br>II. MD rich in ω-3 fatty acids and sterol esters | 8-iso-$PGF_{2\alpha}$ in urine | No effect on 8-iso-$PGF_{2\alpha}$ |
| Buscemi et al.[61], 2009 | 20 overweight/ obese women | RCT,<br>FU: 2 months | I. very -low-carbohydrate diet<br>II. MD | 8-iso-$PGF_{2\alpha}$ in serum | No effect on 8-iso-$PGF_{2\alpha}$ after 2 months. 8-iso-$PGF_{2\alpha}$ significantly only after 5 days in very--low-carbohydrate group |
| Stachowska et al.[65], 2005 | 37 kidney graft recipients | RCT,<br>FU: 6 months | I. MD (21 pt)<br>II. Low fat diet (16 pt) | α-tocopherol in plasma, TBARS in plasma and erythrocytes, and the activities of SOD, catalase and GSH-Px in erythrocytes | In MD group SOD ($p<0.001$ after 6 months), catalase ↓ ($p<0.001$ after 6 months) and GSH-Px ↓ ($P<0.05$ after 2 months), TBARS in plasma ↓ ($p<0.05$ after 6 months), α-tocopherol unchanged |

RCT – randomized controlled trial, FU – follow up, CV- cardiovascular, TMD – traditional mediterranean diet, VOO – virgin olive oil, TAC – total antioxidant capacity, GSH-Px – glutathion peroxidase, MDA – malondialdehyde, oxLDL – oxidized low density lipoprotein, FFQ – food frequency questionnaire, 8-OhdG – 8-hydroxydeoxyguanosine, OS – oxidative stress, 8-iso-$PGF_{2\alpha}$ – prostaglandin $F_{2\alpha}$, TBARS – thiobarbituric acid-reactive species, SOD – superoxide dismutas

lower OS. This association was not confounded by conventional risk factors, familial influences or genetic factors.

## Discussion and Conclusion

Recent evidence shows the positive impact of MD on OS, but data is still scarce. Results from smaller size trials are inconsistent, while bigger size trails are missing. The only available big, longer term RCT contribute to the understanding of the influence of MD on antioxidative capacity, while shorter term RCT showed the influence of MD on oxLDL, both in high CV risk patients. However, data for other patient groups is still inconclusive.

In this review, we explored the problems one could experience when designing the study that aims to explore the effect of MD on OS. First of all, in clinical practice and epidemiological or RCTs, an ideal method of measuring OS is not currently available. In order to get the most out of the presently available techniques it's important that they are used intelligently[69].

Furthermore, in all of the diseases mentioned in this review, more pathways are involved in their pathology besides OS and usually there is interaction between some of them, such as OS and inflammation in atherosclerosis, diabetes or obesity. Therefore, it makes it difficult to measure MD contribution to only the antioxidant mechanism. Our recommendation for the future research would be to involve more parameters in order to account for their interactions. Also, controversy exists whether the increased OS is merely associative rather than causal in those diseases and makes it difficult to differentiate between the preventive and therapeutic effect of MD on OS. Therefore, both epidemiological and randomized controlled trials are needed, and should be tailored to explore the influence of MD in different stages of the diseases.

Other complicating factors are different food components, involved in the diet, that could be responsible for antioxidant properties, and it is very hard to tailor the best diet, both during the research and in the clinical practice. Therefore, scientists should agree on the standard MD to be used in the research. Also, the methods for measuring compliance to diet are matter of scientist's discussion, but that is not in the scope of this review.

Moreover, other influences, such as physical activities contribute to OS and their contribution was rarely accounted in such trials.

Most of the larger studies were done in Mediterranean countries (Greece and Spain)[65–67] and one could argue that the applicability of the results to non-Mediterranean countries could be questioned. Therefore, there is need for multinational randomized trials in the future.

Altogether, there is need for larger, long-term randomized trials in different patient groups and future work should be thoughtfully designed to overcome some of the problems mentioned.

## Recommendations for further reading:

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## ANTIOKSIDATIVNO PROTEKTIVNO DJELOVANJE MEDITERANSKE DIJETE

## S A Ž E T A K

Nedavna meta analiza je pokazala da pridržavanje mediteranskoj dijeti značajno smanjuje rizik od ukupne smrtnosti, smrtnosti od kardiovaskularnih bolesti i karcinoma, te pojavnost Parkinsonove i Alzheimerove bolesti. Kako se sve spomenute bolesti dovode u vezu sa oksidacijskim stresom, mediteranska prehrana sa svojim antioksidativnim učinkom, danas dobiva sve veću pozornost. Danas postoji mnogo istraživanja u tom području, koja većinom upućuju na antioksidativno, protektivno djelovanje mediteranske preherane. Usprkos tome, među postojećim dokazima postoji dosta proturiječnosti. Cilj ovog rada bio je proučiti studije koje su se bavile utjecajem mediteranske prehrane na oksidacijski stress, te ustanoviti područje od značaja za dalja istraživanja.