Translational Section

The Mediterranean Diet

Review in Depth

Health Benefits of the Mediterranean Diet: Metabolic and Molecular Mechanisms

Valeria Tosti, MD,¹ Beatrice Bertozzi, PhD,¹ and Luigi Fontana, MD, PhD¹,²

¹Department of Medicine, Division of Geriatrics and Nutritional Science, Washington University, St. Louis, Missouri. ²Department of Clinical and Experimental Sciences, Brescia University Medical School, Italy.

Address correspondence to Luigi Fontana, MD, PhD, Washington University School of Medicine, 4566 Scott Avenue—Campus Box 8113, St. Louis, MO 63110. E-mail: lfontana@wustl.edu

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Abstract

Consuming a Mediterranean diet rich in minimally processed plant foods has been associated with a reduced risk of developing multiple chronic diseases and increased life expectancy. Data from several randomized clinic trials have demonstrated a beneficial effect in the primary and secondary prevention of cardiovascular disease, type 2 diabetes, atrial fibrillation, and breast cancer. The exact mechanism by which an increased adherence to the traditional Mediterranean diet exerts its favorable effects is not known. However, accumulating evidence indicates that the five most important adaptations induced by the Mediterranean dietary pattern are: (a) lipid-lowering effect, (b) protection against oxidative stress, inflammation and platelet aggregation, (c) modification of hormones and growth factors involved in the pathogenesis of cancer, (d) inhibition of nutrient sensing pathways by specific amino acid restriction, and (e) gut microbiota-mediated production of metabolites influencing metabolic health. More studies are needed to understand how single modifications of nutrients typical of the Mediterranean diet interact with energy intake, energy expenditure, and the microbiome in modulating the key mechanisms that promote cellular, tissue, and organ health during aging.

Keywords: Mediterranean diet, Cardiovascular disease, Cancer

Mediterranean diet is the generic name of the traditional dietary patterns of the individuals living in the Mediterranean region. Historically, in many but not all of the 22 countries bordering the Mediterranean Sea, a great abundance and diversity of nonstarchy vegetables, minimally processed whole-grain cereals, legumes, nuts, and seeds were staple foods for both men and women (Table 1). Unlike in North America and Europe, meat, fish, milk, cheese, and eggs were luxurious foods. For example, in Southern Italy in the 1950s, very little meat was eaten, typically only once every week or two, and milk was never used except in coffee (caffé macchiato) or for infants. Sugar and white potatoes were consumed only in very small quantities, and butter or cream were never used. Cold pressed extra-virgin olive oil was the principal source of fat. Fruits and very small amounts of local cheese were rather regularly consumed, together with a moderate intake of red wine during meals (1,2).

The problem is that since 1950s, the composition of the Mediterranean diet has changed dramatically, and the quality and quantity of food people eat nowadays, for example in Italy, Greece and Spain, has little to do with the Traditional Mediterranean diet (3). Consistently the incidence of coronary heart disease and certain cancers, which was very low in those countries, has increased substantially (1,2,4). Probably, other lifestyle factors, such as a dramatic increase in sedentary lifestyle, excessive calorie intake, psychological stress and pollution might have contributed to the increased incidence of these and other chronic diseases in all these Mediterranean countries (5). However, accumulating data from a combination of epidemiological, human clinical trials, animal and molecular studies indicate that diet remains a key factor in the prevention of cardiovascular disease, obesity, type 2 diabetes and some of the most common types of cancer (6). The purpose of this article is to review...
succinctly the current knowledge on the effects of a Mediterranean dietary pattern on disease risk, and to discuss what is known about its metabolic and molecular adaptations.

**Epidemiological Evidence**

Several population-based and prospective epidemiological studies have shown that adherence to a Mediterranean diet might have a protective effect against cardiovascular disease, stroke, obesity, diabetes, hypertension, several types of cancers, allergic diseases and, most recently, Alzheimer and Parkinson’s disease (7–18). In a large epidemiological study, involving 22,043 men and women, higher adherence to a traditional Mediterranean diet was associated with a significant lower total, cardiac, and cancer mortality, independently of the individual dietary components (19). In another study of 2,339 European men and women aged 70 to 90 years, adherence to a Mediterranean diet was associated with a 23% lower rate of all-causes mortality (20).

In these studies, the adherence to the Mediterranean diet was calculated based on a dietary score, which integrated relatively high intakes of whole-grain cereals, beans, vegetables, fruits, nuts, fish, and monounsaturated fat oils; relatively low intakes of meat, including poultry, and dairy products; and moderate consumption of alcohol. However, we need the bear in mind that epidemiological studies are by nature observational rather than experimental, and the observed associations do not imply a cause–effect relationship.

**Findings From Randomized Clinical Trials**

The first randomized clinical trial showing a protective effect of a Mediterranean-style diet against major cardiovascular events (i.e., coronary recurrence rate after a first myocardial infarction) was the Lyon Diet Heart Study. In this randomized secondary prevention trial, 605 men and women who had suffered from a prior myocardial infarction were randomly assigned to the American Heart Association Step I diet or a diet resembling the Mediterranean diet, supplemented with individual dietary components (19). In another study of 2,339 European men and women aged 70 to 90 years, adherence to a Mediterranean diet was associated with a 23% lower rate of all-causes mortality (20). In these studies, the adherence to the Mediterranean diet was calculated based on a dietary score, which integrated relatively high intakes of whole-grain cereals, beans, vegetables, fruits, nuts, fish, and monounsaturated fat oils; relatively low intakes of meat, including poultry, and dairy products; and moderate consumption of alcohol. However, we need the bear in mind that epidemiological studies are by nature observational rather than experimental, and the observed associations do not imply a cause–effect relationship.

**Potential Metabolic and Molecular Mechanisms Mediating the Effects of the Mediterranean Diet**

The exact mechanism by which a traditional Mediterranean diet exerts its beneficial effects in lowering the risk of developing cardiovascular disease, certain cancers, and other metabolic conditions is not known.
Many interrelated and overlapping factors have been hypothesized to play a role (Figure 1). The five most important mechanisms, which can mediate the prohealth and prolongevity effects of the traditional Mediterranean diet, are as follows: (a) lipid-lowering effect, (b) protection against oxidative stress, inflammation, and platelet aggregation, (c) modification of hormones and growth factors involved in the pathogenesis of cancer, (d) inhibition of nutrient sensing pathways by specific amino acid restriction, and (e) gut microbiota-mediated production of metabolites influencing metabolic health.

Lipid-Lowering Effect

Cardiovascular disease is the leading cause of death for both men and women. Hypercholesterolemia is one of the key risk factors in promoting atherosclerosis, which is the underlying cause of approximately 90% of cases of myocardial infarction, 60% of strokes, most cases of chronic heart failure, peripheral arterial disease, and vascular dementia (31). Plasma low-density lipoprotein (LDL) cholesterol concentration in monkeys and newborn humans are typically lower than 60 mg/dL, and LDL-cholesterol levels higher than 100 mg/dL are common only in people consuming Western diets rich in saturated fat from animal origin (32). Population-based and prospective epidemiological studies have shown that reduced intake of saturated fat is associated with lower plasma cholesterol levels and reduced incidence of coronary heart disease, especially when saturated fat is involved in the pathogenesis of cancer, (d) inhibition of nutrient sensing pathways by specific amino acid restriction, and (e) gut microbiota-mediated production of metabolites influencing metabolic health.

Figure 1. The effectors of the Mediterranean Diet, including reduced saturated fatty acid intake, reduced amino acid and calorie intake, increased phytochemical intake, and microbiota derived metabolites.
Protection Against Oxidative Stress, Inflammation, and Platelet Aggregation

The traditional Mediterranean diet, which includes high consumption of vegetables, whole grains, legumes, fruits, nuts, seeds and extra-virgin olive oil, and moderate intake of red wine, is very rich in antioxidant vitamins (β-carotene, vitamin C, vitamin E), natural folate, phytochemicals (flavonoids), and minerals such as selenium. For example, the total mean dietary beta-carotene equivalents (derived from provitamin A carotenoids), vitamin E (total α-tocopherol), natural folate, flavonoid, and selenium intake of a traditional Mediterranean diet is approximately 6,000 µg/day, 17 mg/day, 400 µg/day, 300 µg/day, and 120 µg/day, respectively.

Increased oxidative stress has been implicated in the pathogenesis of cardiovascular disease, cancer, and many other chronic conditions including dementia. Data from a large case-control study (INTERHEART study) suggest a beneficial effect of dietary antioxidants against coronary heart disease (46). Inadequate intake of dietary antioxidants may increase the risk of developing atherosclerotic plaques because of modifications in lipoprotein oxidation. Plasma levels of oxidized LDL predict acute coronary heart disease in relatively healthy individuals and in patients with coronary heart disease, and is a prognostic marker for subclinical atherosclerosis (47). In a recent randomized clinical trial, individuals randomized to a Mediterranean diet supplemented with extra-virgin olive oil had a significant reduction in circulating oxidized LDL and inflammatory markers (48,49). Oxidative and inflammatory damage are strictly interrelated, and both are instrumental in the pathogenesis of endothelial dysfunction, which represent a key early step in the pathogenesis of atherosclerosis.

Which foods or nutrients of the Mediterranean diet are responsible for the anti-inflammatory effect is not known, but accumulating data suggest that multiple nutrients from a range of diverse foods (and not only few specific ones) have synergistic and interactive roles in reducing inflammation. Indeed, the effect of distinct dietary components may be too small to be detect but their additive impact may be large enough to discern. In support of this hypothesis, data from several epidemiological studies suggest that people consuming higher quality diets have lower inflammation, independently from the classical cardiometabolic risk factors (49,50). Nonetheless, some foods and nutrients have been shown to increase inflammation independently. For example, trans fatty acid intake has been associated with elevated inflammatory markers and an increased risk of developing type 2 diabetes (51,52). In contrast, omega-3 fatty acid intake is inversely correlated with circulating inflammatory markers and triglycerides levels. The anti-inflammatory effects of omega-3 fatty acids seems to be mediated by binding to the G-protein–coupled receptor 120 and inhibition of NLRP3 inflammasome activity (53,54).

Several phytochemicals found in whole grains and extra-virgin olive oil may be responsible for some of their anti-inflammatory and antioxidant effects (55). The aleuron layer of wheat contains a number of phytochemicals (i.e., ferulic acid, alkylresorcinols, apigenin, lignans, and phytic acid), which have antioxidant and anti-inflammatory potential, and anticarcinogenic activities in rodent animal models of colon and skin cancer (56–59). The total phenolic acid content of whole wheat flour ranges from 71 to 87 mg/g, of which more than 80% is accounted by ferulic acid (56,57). Moreover, the germ of whole grains contain a polyamine, called spermidine, which has been shown to extend chronological life span in flies, nematodes, rodents, and human cells (60). Spermidine is known to inhibit histone acetyltransferases, which results in higher resistance to oxidative stress, to increase autophagy as well as to markedly reduce subclinical inflammation and the rates of cell necrosis during aging (60). One hundred grams of extra-virgin olive oil (which is about seven table spoons) contains up to 25 mg of α-tocopherol and 1–2 mg of carotenoids, which are both potent antioxidants, as well as 20–500 mg of oleuropein and 98–185 mg of phytosterols (55). Furthermore, it has been shown that 50 g of newly pressed extra-virgin olive oil contains up to 9 mg of olechanta, a phytochemical with ibuprofen-like COX-inhibitory activity (61). This dose is not sufficient to exert, by itself, a powerful anti-inflammatory effect, but it might be high enough to produce a protection against platelet aggregation and coronary thrombosis. The intake of ibuprofen and aspirin has also been associated with a reduction in the risk of developing cancer, in particular colon cancer, and possibly Alzheimer’s disease because of preferentially reduced secretion of the highly amyloidogenic, Abeta42 peptide (62,63). Nonetheless, we have to keep in mind that one tablespoon of olive oil contains approximately 120 kcal. If we overconsume olive oil, without balancing out with the proper amount of physical exercising, we will gain weight. The effects of overweight and of the excessive accumulation of abdominal fat on chronic inflammation, oxidative stress, insulin sensitivity, and metabolic health in general will overcome the beneficial effects of the polyphenols contained in olive oil (64).

Modification of Hormones and Growth Factors Involved in the Pathogenesis of Cancer

Calorie restriction without malnutrition has been shown to be extremely effective in cancer prevention in rodents and monkeys, and in humans results in major reductions of several metabolic and hormonal factors implicated in the pathogenesis of numerous common cancers and in the biology of aging itself (65). Although consuming a Mediterranean diet does not require one per se to count calories and intentionally lower energy intake, data from randomized clinical trials indicate that substituting refined and processed (high glycemic index) foods typical of the Western diet pattern with minimally processed plant foods representative of the Mediterranean diet results in significant weight loss. For example, in a 5-month study, women randomized to an ad libitum modified Mediterranean diet lost almost 4 kg (66). It has been hypothesized that the short-chain fatty acids produced by the gut microbiota metabolism of the great bulk of resistant starch and oligosaccharides present in the Mediterranean diet can induce satiety by inhibiting gastric emptying through the increased production of gut hormones, such as glucagon like peptide-1 (GLP-1) and peptide-YY (PYY) (67). Importantly, the individuals randomized to the ad libitum modified Mediterranean diet not only lost a significant amount of body weight, but experienced a substantial reduction in fasting glucose and C-peptide, in the area under the curve for insulin, and in total and free testosterone (66). Moreover, the women on the Mediterranean diet had a significant elevation of the plasma concentration of several binding proteins, such as IGFBP-1, IGFBP-2, and SHBG, resulting in a reduction of the biological activity of insulin-like growth factor 1 (IGF-1), testosterone, and estradiol (66). Insulin, estrogens, androgens, and IGF-1 are powerful mitogens for cells, and stimulate the development and growth of several common tumors, including breast, colon, prostate, pancreatic, and endometrial cancer (68). Whether or not these endocrine modifications are due to changes in the quality of diet, to weight loss, or both, is not clear yet. Most likely, the reduction in body fat induced by this low-energy density high-fiber Mediterranean diet...
explains most of the improvement in insulin sensitivity, because the beneficial effects on insulin were no longer statistically significant after adjustment for changes in weight and/or waist circumference (66). However, it is possible that the low-glycemic index, the high intake of monounsaturated and n-3 fatty acids, and the lower intake of branch-chain amino acids may exert some additional beneficial effects in reducing insulin resistance and compensatory hyperinsulinemia (69,70). Moreover, it has been shown that the high-fiber content of the traditional Mediterranean diet can enhance fecal mass and the excretion of estrogens, resulting in reduced plasma concentrations of estrone and estradiol (71). Additionally, a high-fiber diet may directly protect against colon cancer, the second most frequent tumor in Western countries, by accelerating colonic transit and by sequestering and therefore limiting the absorption of carcinogenic substances. Finally, some of the plant foods typically consumed in the Mediterranean diet contain a wealth of chemical compounds with other potential health benefits against cancer, including lycopene (tomato); capsaicin (hot pepper); organosulfur compounds (onions, garlic); isothiocyanates, indol-3-carbinol, sulforaphane (cabbage); pycnogenol (pumpkin, carrots); monoterpenes (oranges, lemons); ginkgetin (capers); and ferulic acid and spermidine (whole grains). In particular, formononetin, biochanin A, paeoniflorin, genistein, and daidzein (found in beans and in particular cabbage, broccoli, and cauliflower), can inhibit endogenous estrogens in binding to the estrogen receptors, and thus blocking its mitogenic effects (72).

Amino Acid Restriction Induced Inhibition of Nutrient Sensing Pathways

Total protein intake in the traditional Mediterranean diet is on average 20% lower than in typical Western diet (e.g., 90 vs 70 g/day), with animal protein consumption being 50 to 60% lower (e.g., 30 vs 70 g/day). Most of the protein comes from legumes and whole cereals; the average daily vegetable protein content of the traditional Mediterranean diet is approximately 40 g. This is important because accumulating data indicate that moderate protein restriction extends life span, independently of calorie intake, in multiple model organisms, including rodents (73). Moreover, it has been shown that isocaloric restriction of protein or substitution of plant for animal proteins markedly inhibits prostate and breast cancer growth in human xenograft animal models of cancer, with reduced serum IGF-1 levels and downregulation of the mechanistic target of rapamycin (mTOR) activity in the tumor and normal tissue as well (74).

In a recent epidemiological study, individuals aged 50–65 years with the highest protein intake (more than 20% of calories from protein) had a 75% increase in total mortality and a fourfold increase in cancer mortality. These associations were either eliminated or diminished if people were consuming plant-based proteins (75). Moreover, data from several epidemiological studies suggest that high protein intake is associated with an increased risk of obesity, cardiovascular disease, and type 2 diabetes (76). The risk of developing type 2 diabetes, for example, increases by 20–40% for every 10 g of protein consumed in excess of 64 g/day (76,77). Accordingly, in an elegantly designed clinical trial, protein supplementation of a calorie-reduced weight loss diet prevents the improvement in insulin-mediated glucose disposal induced by 10% weight loss in women with obesity (78). In contrast, in another randomized clinical trial, feeding customized, isocaloric, moderately protein-restricted diets for 4–6 weeks to middle-aged overweight and mildly obese men resulted in a significant decrease in body weight, fat mass, and fasting blood glucose levels, and a major increase in circulating FGF21 concentration (79).

On the other hand, it is likely that protein quality may be more important that quantity in mediating the beneficial effects of the Mediterranean diet. Because of the different ratio of animal to vegetable protein, the content of some essential amino acids is much different between the Western and the Mediterranean diet. This is important because multiple model organisms, including rats and mice, dietary methionine restriction has consistently been shown to extend average and maximal life span, and protect against multiple chronic disease, in particular cancer (80). In rodents, methionine restriction improves glucose metabolism, protects against obesity and hepatic steatosis, and reduces oxidative stress. In addition, methionine restriction induces plasma elevations of adiponectin and FGF21, and reduces serum IGF-1, T4, and leptin concentration, which are all hormonal adaptations induced in long-lived rodents by calorie restriction as well (80).

Not only methionine but also the intake of other essential amino acids, such as leucine, isoleucine, valine, and tryptophan, is 20 to 30% lower in the traditional Mediterranean diet than in the meat, egg, and dairy rich Western diet. Interestingly, accumulating data indicate that the branched-chain amino acids, leucine, isoleucine, and valine, play a key role in modulating insulin sensitivity (81). The circulating concentrations of branched-chain amino acids are elevated in insulin resistant humans and rodents, and high consumption of leucine, isoleucine, valine is associated with a 11–13% increased risk of type 2 diabetes in three large prospective cohort studies (81). In contrast, data from a recent rodent study show that selectively reducing the dietary intake of branched-chain amino acids significantly improves glucose tolerance, β cell metabolic stress, and body composition (79).

At the cellular level, the availability of essential amino acids is sensed by multiple nutrient sensing pathways, the most important being mTOR and GCN2. The activation of the pro-aging and pro-cancer mTOR pathways is modulated by energy intake but also by a cocktail of different essential amino acids, with the branched-chain amino acids leucine and isoleucine playing a fundamental role (82). Dietary, genetic, and pharmacological (rapamycin) inhibition of mTOR extends life span in multiple model organisms (83). In contrast, the restriction of individual amino acids upregulates the GCN2 pathway, which stabilizes ATF4, a transcription factor indispensable for the Integrated Stress Response (84).

Gut Microbiota-Mediated Production of Metabolites Influencing Host Biology

Diet has a major impact on gut microbiome biology (85). Accumulating metagenomic data show that specific nutrients, especially protein and insoluble fiber, have profound effects on gut microbiota structure, function, and secretion of metabolites that modulate immune function and multiple metabolic and inflammatory pathways (86–88). For example, the traditional Mediterranean diet content of choline and L-carnitine, which are abundant in red meat, eggs, and cheese, is more than 50% lower than in a typical Western diet. Recently, it has been shown that gut microbial production of trimethylamine N-oxide (TMAO) from dietary choline and L-carnitine increases the risk of developing cardiovascular disease in both mice and humans, independently of traditional cardiometabolic risk factors (89). Elevated level of TMAO induces vascular inflammation and
a direct prothrombotic effect by increasing platelet hyper-responsiveness to multiple agonists in both rodents and humans, and might be involved in the pathogenesis of obesity and type 2 diabetes (90,91).

Another major characteristic of the Mediterranean diet is its very high content and bioavailability of fiber, and in particular of insoluble fiber, which is more than 2-fold higher than in a usual Western diet (30 vs 14 g/day). It has been shown that high dietary fiber intake promotes modifications of the gut microbiota in both rodents and humans, with decreased Firmicutes and increased Bacteroidetes (in particular *Bacteroides acidifaciens*), which produces high levels of short-chain fatty acids, including acetate, propionate, or butyrate. Accumulating experimental animal data indicate that gut microbial production of these short-chain fatty acids from dietary fiber can suppress the development of several inflammatory, autoimmune, and allergic disease (92). Some of the beneficial effects of these microbiome-derived metabolites are thought to be mediated by the binding to specific G-protein-coupled receptors expressed on enteroendocrine and immune cells (92).

Consistently, in a recent randomized clinical trial, obese individuals randomized to a Mediterranean diet for 2 years experienced a reshaping of the gut microbiota, with an increase in Bacteroidetes, Prevotella, and Faecalibacterium genera, and most importantly of the Roseburia and *Ruminococcus* genera and *Parabacteroides distasonis* and *Faecalibacterium prausnitzii* bacterial species, which are known for their saccharolytic activity and the capacity to metabolize carbohydrates to short-chain fatty acids (93). Recently, it has been shown that *Bacteroides fragilis* and *F. prausnitzii* are instrumental in inducing CD4+ T cells that secrete the anti-inflammatory interleukin-10 (94,95). In another study, a high adherence to the Mediterranean dietary pattern characterized by a high intake of vegetables, legumes, and fruit was associated with an enrichment of Firmicutes and Bacteroidetes and a rise in fecal short-chain fatty acid levels. In contrast, a poor adherence to the Mediterranean diet was associated with increased *Ruminococcus* and Streptococcus bacteria, and higher urinary TMAO concentration (96).

It is likely that a long-term adherence to a certain dietary pattern (e.g., Mediterranean diet rich in minimally processed plant foods) may have a more profound impact on the composition and diversity of taxa maintained within the gut microbial community than short-term dietary modifications. Indeed, long-term consumption of plant-rich diets with restricted caloric intake has been associated with richer and more phylogenetic diverse fecal microbiota (97). In contrast, multigenerational exposure to a Western diet poor in “microbiota-accessible carbohydrates” result in the extinction of specific bacterial lineages, which might negatively influence the maturation and function of the immune system, and increase the risk of developing a range of metabolic, inflammatory, allergic, and autoimmune diseases (92,94,98). This is important because accumulating evidence suggest that reprogramming human gut microbial functions through long-term adherence to healthier plant-rich diets may influence the physiologic response to specific nutrients, to caloric restriction and to other features of host biology that are instrumental in promoting health and longevity (97,99).

**Conclusions**

Accumulating data strongly indicate that nutrition is a key factor for the promotion of health and the prevention of the most common age-associated chronic diseases. Both the quantity and quality of what we eat is essential to promote metabolic and molecular health (6). Calorie restriction extends health span and life span only when coupled with adequate intake of all the essential nutrients and micronutrients (5). The traditional Mediterranean diet, unlike the typical North European and American diet, incorporates a wide range of minimally processed fiber-rich plant foods, packed with vitamins, minerals, and phytochemicals. The low consumption of fish, meat, eggs, and cheese provides other essential nutrients, such as vitamin B12, which are lacking in an exclusive plant-based diet. In the past, the surplus of energy required to perform the high levels of physical labor (approximately 70–80 h of work per week) was provided by the consumption of energy-dense food, such as extra-virgin olive oil, wine, and dried fruits.

Recent findings from animal and human translational studies are starting to shed light on the biological mechanisms that are mediating the beneficial effects of the traditional Mediterranean diet and other healthy dietary patterns such as the traditional Okinawan diet (6). The moderate energy restriction provided by the high consumption of fiber-rich energy-poor plant foods, the specific restriction of sulfur and branch-chain amino acids and saturated fatty acids, seems to play prominent roles in mediating the health and longevity effects of these traditional diets. The gut microbiome processing of a multitude of plant foods packed with fiber, a wide range of vitamins and phytochemicals, can also play a key role in promoting metabolic and molecular health. However, more mechanistic studies are needed to understand the interactions among caloric intake, single-nutrient modifications, the microbiome, and physical exercise in modulating the key molecular pathways that promote cellular, tissue, and organ health during aging.

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**Conflict of Interest**

None reported.

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