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Editor's Choice

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, PhD[1,2]

[1]Department of Medicine, Division of Geriatrics and Nutritional Science, Washington University, St. Louis, Missouri. [2]Department 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

Table 1. Dietary Characteristics of the Traditional Mediterranean Diet

Mediterranean diets
1. A variety of minimally processed whole grains and legumes as the staple food
2. Plenty of a huge diversity of fresh vegetables consumed on a daily basis
3. Fresh fruits as the typical daily dessert; sweets based on nuts, olive oil, and honey consumed only during celebratory occasions
4. Cold pressed extra-virgin olive oil, nuts and seeds as the principal source of fat
5. Moderate consumption of fish
6. Dairy products (mainly local cheese and yogurt) consumed in low amounts; butter, cream and milk never used, except for milk in coffee (caffé macchiato) or for infants
7. Red and processed meat consumed in very low frequency (only once every week or two) and amounts;
8. Wine consumed in low to moderate amounts only with meals

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 the Mediterranean diet might have a protective effect against cardiovascular disease, stroke, obesity, diabetes, hypertension, several type 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 two servings per day of a margarine rich in α-linolenic acid. Patients randomized to the "Mediterranean-style diet" were instructed to consume more bread, vegetables, fruit, and fish, and less meat that was replaced with poultry, while butter and cream were exchanged with margarine high in α-linolenic acid. After a 27-month average follow-up, the trial was stopped early because the Mediterranean diet group had a significant 70% reduction in all-cause mortality due to a 73% reduction in coronary heart disease mortality and analogous major reductions in nonfatal complications (21). Indeed, despite a similar cardiometabolic risk profile, there were 16 cardiac deaths in the control and 3 in the experimental "Mediterranean α-linolenic acid–rich diet" group; 17 nonfatal myocardial infarction in the control and 5 in the experimental groups; overall, mortality was 20 in the control, 8 in the intervention group (21). Importantly, the beneficial effect of the "Mediterranean α-linolenic acid–rich diet" was maintained up to 46 months after the first cardiac event, confirming the previous intermediate analyses of a striking protective effect against the recurrence of heart disease, as measured by three different combinations of outcome measures including (i) cardiac death and nonfatal heart attacks; (ii) the preceding plus unstable angina, stroke, heart failure, and pulmonary or peripheral embolism; and (iii) all of these measures plus events that required hospitalization (22).

In another randomized clinical trial, Singh and colleagues tested a "Indo-Mediterranean diet," rich in whole grains, fruits, vegetables, walnuts, almonds, mustard, or soybean oil, in 1,000 Indian patients at high risk or with existing coronary heart disease, and found a significant reduction in total cardiac end points. Compared with patients randomized to a step I National Cholesterol Education Program prudent diet, those following the Indo-Mediterranean diet rich in α-linolenic acid experienced an approximate 50% reduction in the rate of nonfatal myocardial infarction and an approximate 60% reduction in the rate of sudden cardiac death (23).

The PRIDIMED study was a primary prevention randomized trial on the effects of a Mediterranean diet, supplemented with approximately 1 liter per week of extra-virgin olive oil or 30 g of mixed nuts per day, conducted in 7,447 men and women at high cardiometabolic risk, but with no evident cardiovascular disease at baseline. After a median follow-up of 4.8 years, a total of 288 primary-outcome events occurred, of which 83 in the group randomized to a Mediterranean diet supplemented with nuts, 96 in the group randomized to a Mediterranean diet with extra-virgin olive oil, and 109 in the control group (24). The absolute risk reduction was of about three major cardiovascular events per 1,000 person-years, for a relative risk reduction of approximately 30%. However, among the components of the combined primary end point, only the comparisons of stroke risk reached statistical significance, but not myocardial infarction alone. Using the same database, this Spanish team of researchers published a number of substudies (secondary analysis) addressing other chronic conditions. The incidence of type 2 diabetes, peripheral artery disease, atrial fibrillation, breast cancer, but not the incidence of heart failure, was significantly reduced in individuals randomized to the Mediterranean diet (25–27). Other intervention trials using the Mediterranean diet have also shown some beneficial effects in the treatment of obesity, the metabolic syndrome, and arthritis (28–30).

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.

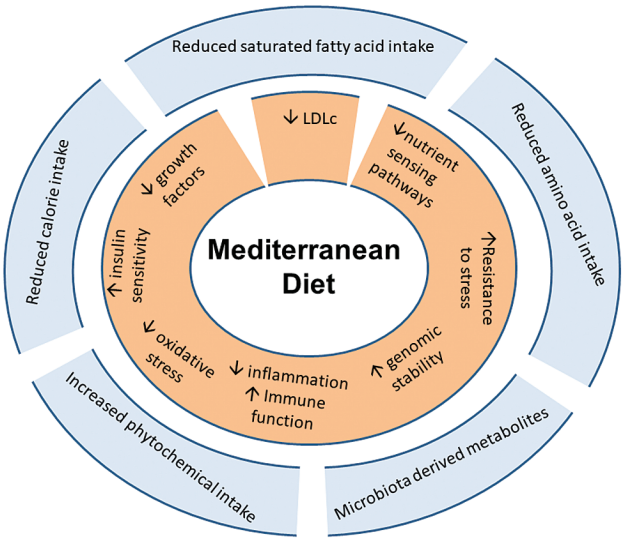

Figure 1. The effectors of the Mediterranean Diet, including reduced saturated fatty acid intake, reduced amino acid and calorie intake, increased phytochemical intake, and microbiota derived metabolites.

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 substituted with polyunsaturated and monounsaturated fat (33). It has been estimated that substituting 5% of energy intake from saturated fats with an equal amount of energy from polyunsaturated fats, monounsaturated fats, or carbohydrates from whole grains is associated with a 25%, 15%, and 9% lower risk of coronary heart disease, respectively. In contrast, replacing saturated fats with carbohydrates from refined grains is associated with an increased risk of coronary heart disease (34). Several randomized controlled clinical trials have confirmed that replacing dietary saturated fat intake with vegetable polyunsaturated fats reduce cardiovascular disease incidence by approximately 30%, which is similar to the decrease induced by statin's therapy (35). However, the cardiovascular benefits could be much larger if plasma cholesterol would be kept lower throughout the entire life, thus preventing the development of the atherosclerotic plaques from the beginning. Indeed, individuals heterozygous for mutations that inactivate one copy of the PCSK9 gene have plasma LDL-cholesterol levels that are approximately 30% lower (which are similar to those induced by treatment with statins), and experience a 90% reduction in heart attacks, independently of smoking, hypertension, and diabetes (36). Similarly, a lifelong reduction of only 11% in plasma LDL-cholesterol levels due to loss-of-function mutations in NPC1L1, an intestinal cholesterol transporter, causes a striking 53% reduction in myocardial infarction (37). These data suggest that lifelong lower levels of plasma LDL cholesterol are much more effective than late LDL reductions induced by statins in preventing coronary heart disease (31). Moreover, they suggest that the extremely low incidence of coronary heart disease observed by Ancel Keys and others in South Italy, Crete, Japan, and Okinawa in the 1950s was probably due, at least in part, to the lower levels of plasma LDL cholesterol that these populations had experienced throughout life (1,2).

Because of the very low consumption of meat, milk, and butter, the intake of saturated fat in the traditional Mediterranean diet is low (approximately 8% of energy), despite a relative high intake of total fat (25% to 35% of calorie) coming predominantly from extra-virgin olive oil, a wide variety of nuts, seeds, and the germ of whole grains. Nuts, in particular almonds, walnuts, hazelnuts, and pine nuts, are a very good source of omega-6 and omega-3 fatty acids and plant sterols, which might contribute in lowering LDL-cholesterol and coronary heart disease risk. Prospective studies have shown that eating 5 servings of nuts per week is associated with a 40% to 60% decrease in coronary heart disease events (38,39). In a 6-month randomized clinical trial, the consumption of a range of cholesterol-lowering foods (i.e., nuts; soy protein; viscous fibers from oats, barley, and psyllium; plant sterol ester–enriched margarine) resulted in a significant 13% reduction of plasma LDL cholesterol (40).

Additional mechanisms can explain the lower levels of plasma cholesterol in individuals eating a Mediterranean diet. A typical traditional Mediterranean diet rich in whole grains, legumes and dried fruits provides at least 14 g of vegetable fiber for every 1,000 kcal per day, which is more than double what is consumed every day in many industrialized countries. In particular, data from randomized controlled studies indicate that high consumption of water-soluble fibers (which are found in high concentrations in beans and fruits) has a significant cholesterol-lowering effect; each additional gram of water-soluble fiber in the diet lowers plasma LDL cholesterol concentrations by approximately 1.12 mg/L (41,42). It has been hypothesized that water-soluble fiber reduces the (re)absorption of cholesterol and bile acids in the small intestine, thus resulting in an augmented LDL uptake by the liver (42). In addition, low-glycemic foods rich in dietary fiber have been shown to lower insulin production and increase the levels of short-chain fatty acids produced by fiber fermentation, which have both been demonstrated to inhibit cholesterol synthesis (42). The high intake of phytosterols from nuts, seeds, whole grains, vegetables, and fruits may also play a significant role in lowering plasma cholesterol levels by competing with intestinal cholesterol absorption (43).

Finally, in the past, the traditional Mediterranean diet was also extremely low in partially hydrogenated trans fatty acids, which are important factors in the pathogenesis of coronary heart disease. Substituting calories from mono- or poly-unsaturated fat with trans fatty acids increases LDL cholesterol, apolipoprotein B, triglycerides, and lipoprotein(a), and lowers plasma high-density lipoprotein cholesterol and apolipoprotein A1 levels (44). Accumulating data suggest the naturally occurring trans fatty acids found in milk and meat of ruminant animals have similar adverse effects on blood lipids (45).

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 mg/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 bran contains a number of phytoprotectants (i.e., ferulic acid, alkylresorcinols, apigenin, lignans, and phytic acid), which have antioxidative 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 tablespoons) 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 olechantal, 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 (onion, garlic); isothiocyanates, indol-3-carbinol, sulforophane (cruciferous vegetables); polyacetylenes (pumpkin, carrots); monoterpenes (oranges, lemons); ginkgetin (capers); and ferulic acid and spermidine (whole grains). In particular, formononetin, biochanin A, coumestans, genistein, and daidzein (found in beans and in particular fava beans and lupin), which are low-potency estrogenic molecules (i.e., phytoestrogens), can compete with 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. For example, dietary methionine intake is on average 40% lower in the traditional Mediterranean diet. This is important because in 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 Bacteroides, 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 associates 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 l-*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 calorie 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 prolongevity 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 calorie 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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