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Essays

Translating the Mediterranean diet: from chemistry to kitchen

JENNIFER PARISH[1]
MIHAELA PERIĆ[2]
HANA ČIPČIĆ PALJETAK[2]
MARIO MATIJAŠIĆ[2]
DONATELLA VERBANAC[2#]

[1]University of Zagreb, School of Medicine Šalata 3, 10000 Zagreb

[2]University of Zagreb, School of Medicine, Center for Translational and Clinical Research, Šalata 2, 10000 Zagreb

Correspondence:
Donatella Verbanac
University of Zagreb, School of Medicine, Center for Translational and Clinical Research, Šalata 2, 10000 Zagreb
E-mail: donatella.verbanac@mef.hr

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Abstract

The Mediterranean diet has been an area of popular and scientific intrigue for more than 30 years. Research has focused on both whole-diet compliance as well as individual component effects and many questions remain open as to the full spectrum of effects from this olive oil rich eating pattern. This paper summarizes key findings related to the Mediterranean diet including the health effects of olive oil as one tool in a whole-organism approach to modifying risk factors for cardiovascular disease, cancer, metabolic and other chronic illnesses. Suggestions for the utilization of these findings are summarized in a new »Holistic Food Pyramid«, optimized according to the basic principles of the Mediterranean diet and overall metabolic needs.

INTRODUCTION

Biologically active compounds abound at both pharmacy and table with neither food nor drugs being expected to ever be able to completely substitute the other. Although modern pharmaceuticals are commonly accepted as a miracle of the 20th century, we must remember that food remains important to health as well. Dietary intake can enhance our possibilities to heal faster, reduce symptoms and minimize disability.

The popularization of healthy diet design is no new phenomenon and yet the average consumer can easily be left dazzled by the variety of available approaches. Fortunately, medical professionals with a firm understanding of the basic nutritional principles common to any diet regimen can help guide their patients toward their best-suited options based not only on personal preferences but also on the emerging understandings of expected health implications of specific dietary choices. The Harvard School of Public Health, for example, has designed a food pyramid that includes an active lifestyle plus a Mediterranean diet, the same plan recommended by the World Health Organization *(1)*.

This original paper is aimed at defining the components of the Mediterranean Diet, its adherence to basic nutritional dietary regimes and its specific expected health effects.

CHEMISTRY: FOODS AND METABOLISM

All metabolic processes ideally adhere to one basic law: food and oxygen provide for the production of carbon dioxide, water and energy. Half of that energy, in the form of heat, maintains the body temperature

necessary for optimal chemical reactivity. The remainder of the energy provided from food takes the form of energy-dense compounds such as ATP. Worth remembering is that oxygen plays a vital role in allowing the optimal processing of foodstuffs and that a deficiency of oxygen, hypoxia, is a contributing step toward inflammation *(2)*.

Essential dietary intake not only includes the macronutrients composed of proteins, carbohydrates and lipids but also micronutrient minerals and vitamins. Nutritional supplements ensure a complete intake of micronutrients *(3)* despite their dwindling quantities in foods due to soil depletion and food processing *(4)*. Water, the milieu required for all bodily reactions, is one component publicly understood to be part of necessary dietary intake; however, given that it is also a metabolic product, it contributes, in part, to the variability of the precise required daily water intake between individuals *(5)*.

Although these fundamental components remain the same for all humans, precisely how each individual processes these nutrients is effected by the interplay of many components, several on which we can exert direct influence.

METABOLIC REGULATORS

The overall influence on metabolic regulation must be considered with regard to intrinsic, internal, external and epigenetic, contributors.

The intrinsic components include the hypothalamus, the neuroendocrine control centre for most metabolic processes. While hypothalamic function generally remains within predictable ranges, the variable quantity and composition of adipocytes between individuals is most significant in its role as metabolically active tissue. The ancient concept of fat serving only a benign insulation and storage function must forever be abandoned in recognition of its production of leptin, adiponectin and resistin; proteins and peptides which collectively influence the balance of intake and expenditure coordinated by the hypothalamus *(6,7)*.

In addition to the hypothalamus and adipocytes, a third intrinsic component, having been widely accepted as such since the late 1990's publication of »The Second Brain«, is the intestinal tract *(8)*. This massive system of digestion and absorption is connected to metabolic regulation as the location for 90% of the body's serotonin receptors, a neurotransmitter well known to modulate mood, satisfaction, pleasure and affect.

Furthermore, the digestive tract is now understood to be highly influenced by another internal component of metabolic regulation, the metabiome: the sum total of cells, human and otherwise, within the body. No longer simply a shocking concept, the reality of each 'human' body containing only 10% self-cells with the remaining 90% being bacterial must be appreciated for its implications in overall bodily function *(9)*. The vast array of gene products provided by resident microbes is known to diversely supplement the host's own metabolic processes *(10)*. This includes the extraction of energy from dietary components, an association that is, for example, essential with regard to polysaccharides and lipids *(11)*.

The microbial-influenced capacity for energy extraction has been shown in animal models to be correlated to body mass. The ability of enhanced energy extraction is transmissible from obese to lean mice via the inoculation of gut microbes from the former to the latter. That is, germ-free lean mice can be converted to overly-exuberant energy extraction and storage phenotypes by virtue of providing them the gut flora of their obese counterparts *(12)*. Similarly, in humans, colonization with an obesity-associated microbiota appears to precede the accumulation of excess body fat *(12)*. Further influences of the microbiotia include modulation of neurohormones and gut-derived lipids, short-chain fatty acids, triglyceride clearance, vitamin biosynthesis, mucosa associated immunity, inflammatory bowel disease and colorectal cancer *(10)*. Such associations make the discussion of diet and health without considerations for the influence of microbiota inherently incomplete. Dietary choices from the start of life influence who we will carry with us in our life's journey: bacterial colonization of the gut begins with food intake. We humans are communities of cells living in perfect, functional harmony: not only do we take care of our microbes but they take care of us.

External influences on metabolic regulation include exposure to sunlight. Not only is this the signal for regulation of circadian rhythms via the suprachiasmatic nucleus of the hypothalamus *(6)*, it has been hypothesized as a potential confounder in the analysis of epidemiologic observations of health impacts from the Mediterranean diet *(13)*. Although worth considering, the reproducibility of the health impact of the Mediterranean diet in laboratory and cross-sectional studies worldwide suggests this diet's health impacts are independent of the benefits of sunlight.

Therefore, two groups of non-human systems consisting of internal (metabiome) and external influences (sunlight, food) must be regarded in the spectrum of influences, with food being that with which we interact most often. Considering the liable and various phenotypes possible from such an array of contributors, no one diet could reasonably be expected to suit all needs. All the same, a growing body of evidence suggests that the Mediterranean diet may be a favorable nutritional foundation for many individuals.

CHEMISTRY: DEFINING A MEDITERRANEAN DIET

Epidemiologic evidence has shown a correlation between the dietary habits and health outcomes within the Mediterranean region. In order to study this relationship, however, the Mediterranean diet cannot simply be assumed to be homogenous across geographies nor to remain static over time *(14)*. During a 1993 international conference, the Mediterranean diet was defined as one

which abundantly contains plant-derived, minimally processed foods which include olive oil as the main source of fat, fresh fruits and nuts as the primary deserts, small quantities of dairy products comprised mainly of cheese and yogurt, low to moderate amounts of red wine with meals, less than four eggs per week and red meat only occasionally and in small quantities *(15)*. These dietary components traditionally associated with the Mediterranean region were first brought to the attention of the English-speaking world by American researcher Dr. Ancel Keys *(16)*. Although he's often remembered for having developed K-rations for combat soldiers in World War II, he also conducted pioneering work on the effects of starvation and introduced the concept of the body--mass index (BMI) assessment of body composition. Preceding and during his nearly 50 years of living in southern Italy, Keys published studies and books on the health benefits of Mediterranean diet and life-style *(16)*.

Keys expounded on the important cardiovascular protective factors associated with Mediterranean diet. His claims were based on decades of studying the epidemiology of cardiovascular disease from which he made some of the earliest claims that specific dietary fats correlate to health risks *(17)*. Studies such as the landmark 15-year »Seven Countries Study« utilized habits, vital signs, biochemical and hematological markers to follow the epidemiology of disease in particular regions. With results published between 1970 and 1986, Keys and his team had strong evidence that death rates accelerated with increased intake of saturated fatty acids, while monounsaturated fatty acids were associated with decreased cardiovascular disease and increased longevity *(17, 18)*. Furthermore, in areas where olive oil served as the main source of fat, all-cause cardiovascular disease and mortality rates were low.

Finland, one of the participants in the »Seven Countries Study«, had, in 1970, massive health problems related to a lack of sunlight and an excess of butter, salt and meats. Today, however, having conducted massive studies and implemented strategic interventions, this same nation serves as an international role model for positive health outcomes through progressive public health programs into the modifiable disease affecters associated with diet *(19)*.

The Mediterranean diet should therefore be considered to be inspired by, but not limited to, applications within the Mediterranean region. Although there appears to be synergistic effects between the component ingredients *(20)*, research into the Mediterranean diet has largely centered on the association first noted by Keys: the significance of olive oil as the main source of dietary fat. The intervening 25 years since Keys' first publication have continued to demonstrate a strong correlation between improved cardiovascular profiles and the Mediterranean diet as well as growing public acceptance of these health benefits *(21, 22)*.

CHEMISTRY: COMPONENTS AND THEIR BIOLOGIC EFFECTS

Olive oil

Olive oil has been heavily researched with regard to the activity of its individual components, as well as to their potential synergistic effects when these constituents remain combined in the form of the natural oil. Although many studies have searched for the primary component responsible for the health benefits associated with olive oils, much evidence suggests that these are best explained by the combined effect of olive oil's high monounsaturated fatty acid (MUFA) content along with several classes of bioactive compounds *(23, 24)*.

Oleic acid is the primary fatty acid of olive oil. Although also present in other fats, this MUFA is found in highest relative abundance in virgin olive oils (VOO) *(25)*. Oleic acid has been shown to improve several biophysical parameters including a three-fold positive impact on biochemical contributors to cardiovascular disease. Firstly, oleic acid augments production of high-density lipoprotein (HDL), the »good cholesterol« whose level correlates with protection from cardiovascular disease. Inversely, harmful low-density lipoprotein (LDL), the »bad cholesterol« most closely correlated to the development of atherosclerosis, is meanwhile decreased by oleic acid. Finally, although oxidized LDL enhances atherosclerotic plaque development by promoting monocyte chemotaxis, this process is additionally inhibited when the LDL contains oleic acid *(24)*.

Oleocanthal is the ester responsible for olive oil's pungent flavour. Inspired by the classic medicinal association of bitterness in plants with potential pharmacologic properties, a research team in Barcelona demonstrated the correlation between the intensity of bitterness in olive oil with its anti-inflammatory effects *(24)*. They confirmed that the mechanism of action for oleocathal mimics ibuprofen in that it inhibits the COX-1 and COX-2 enzymes *(26)*. Additive as well as synergic effects on other related physiological functions, such as LDL oxidation and blood pressure, were also shown. Of note is ibuprofen's association with anti-platelet aggregation, decreased cancer and decreased Alzheimer's-associated amyloid production *(24)*, potent benefits which are necessarily unexploited due to high-dose ibuprofen's liver and kidney toxicity *(27)*.

Other authors doubt if the whole dose of olecanthal is bioavailable *in vivo* and have described phenolics as the source of olive oil's anti-inflammatory properties *(24)*. In fact, refined olive oil (ROO) is devoid of many of these biophenols and therefore only virgin olive oil is certain to contain all the components that are likely contributing to the anti-inflammatory properties of olive oil *(24, 28)*.

Biophenols in olive oil increase the oxidative stability, enhance the olive oil's flavor and have been shown to be highly bioavailable *(23)*. This group includes hydroxytyrosol, tyrosol and an oleuropein mixture, all of which are biologically active agents *(29)*. They have been sho-

wn to contribute to the cardioprotective effects of olive oil *(29, 30)*.

Squalene, a component whose abundance in olive oil is uniquely high compared to other fats, has been shown to inhibit the activity of HMG-CoA reductase *(31)*. This key enzyme in the synthesis of cholesterol is the target for the trusted statin class of cholesterol-lowering drugs, as well as some new anti-inflammatory drugs. Additionally, squalene has been shown to exert a protective effect against colon carcinogenesis through its influences on bile synthesis *(32)*. Squalene was previously credited for the decreased incidence of breast cancer observed in certain studies involving dietary olive oil *(25)*. The mechanism of impact on breast cancer requires more research although it now appears to be more likely related to oleic acid *(33)*.

Tocopherols, including the alpha-tocopherol vitamin E, are abundant in olives and olive oil. The ratio of vitamin E and polyunsaturated fatty acids in olive oil is optimal *(34, 35)*. Vitamin E is associated with improving parameters related to nearly every process known to be contributing factor in atherosclerosis. This spectrum of activity includes increasing the resistance of LDL to oxidation, decreasing the cytotoxic effects of oxidized LDL on endothelial cells, inhibiting monocyte release of reactive oxygen species and pro-inflammatory cytokines, inhibiting smooth muscle cell proliferation and increasing the bioavailability of endothelial nitric oxide, to name a few *(36)*. The known anti-inflammatory properties of vitamin E have been linked to observational studies documenting reduced cardiovascular risk; however, a convincing etiologic connection remains to be confirmed *(37)*.

Pigments are often regarded as a helpful visual guide to the micronutrient content of fruits and vegetables. Olives and olive oil contain chlorophyll, a green pigment, which has been suggested to have antioxidant and cancer prevention effects. They also contain carotenoids that were shown to act as provitamins and antioxidants, as well as to have a role in the prevention and treatment of cancer, cardiovascular, skin and eye disorders *(38)*.

Aromatics, in particular trans-2-hexanal, are utilized in the assessment of olive oil quality as they provide the oil its aroma *(22)*. While not generally considered to be important pharmacologically, these compounds enhance the olfactory and therefore gustatory experience of foods.

Red wine

Daily red wine consumption has been shown to have a predictable correlation to mortality risk: one glass is more protective than any other amount, including none *(39)*! The recommended size of a 'glass' varies, with 0.3 – – 0.4L being suggested for men while women should only drink half as much. Furthermore, wine should be taken with meals as alcohol consumed outside of meals leads to the depletion of important coenzymes that can contribute to disease *(40)*.

The health benefits of red wine are, in part, attributed to it being a natural and most concentrated source of reservatrol. This polyphenol activates the sirtuin enzyme SIRT-1 which is believed to promote survival in times of stress *(41, 42)*. This mechanism is credited for the cardioprotective and other health benefits of red wine but there remains debate regarding the bioavailability of reservatrol and the precise therapeutic target in the body *(36, 41, 43)*.

Wine's health benefits have been further shown to extend to the prevention of the formation of the neurofibrillary plaques associated with Alzheimer's disease *(44)*. This has contributed to the pursuit of sirtuins as drug targets for neurodegenerative disorders like Parkinson's and Alzheimer's disease *(42)*.

KITCHEN: LIFE-STYLE AND THE UTILIZATION

Despite the plethora of claims that hold true about the positive effects of components of the Mediterranean diet, the most compelling evidence for their effectiveness comes from the positive correlation between health outcomes and the degree to which one adheres to the Mediterranean diet. That is to say, the simple addition of olive oil to one's meals is likely to decrease the risk for disease development; however, the increased health benefits associated with a strictly Mediterranean diet are more than additive. Using a variety of assessment techniques for quantifying adherence to such a diet, the results have consistently demonstrated reduced morbidity and mortality with increased levels of adherence to the Mediterranean diet *(45)*. All the same, this cannot be considered to be a fully elucidated diet plan as several key nutritional guidelines are lacking, including recommendations about calorie intake and the distribution of macronutrients. In order to address these needs, we require a holistic perspective on the health benefits of the Mediterranean diet, allowing it to provide a natural epidemiologic and scientific foundation for individualizing diet schemes. Our team has summarized these findings, along with nutritional and life-style suggestions, in a new »Holistic Food Pyramid« (Figure 1).

There is no single, generic food model nor can any diet be designed without consideration for its role as part of one's life-style. Therefore, we shouldn't speak only of the Mediterranean diet in general but instead about elements of Mediterranean food and lifestyle. Dietary advice is incomplete if it lacks the recommendation to take care of *How* and *When* to eat, not only what to eat.

The »*How* of eating« suggests a total life-style approach including adequate physical activity, sleep and stress reduction. A life-style of eating well is impossible without consideration for the availability of, and habits involved in, healthy food selection. Although purchasing costs may initially make some hesitant to add high quality olive oils and wines to their regular diet, the daily quantities required for health benefits negate the up--front costs while promising huge gains in life-quality, in part through added food diversity and flavors.

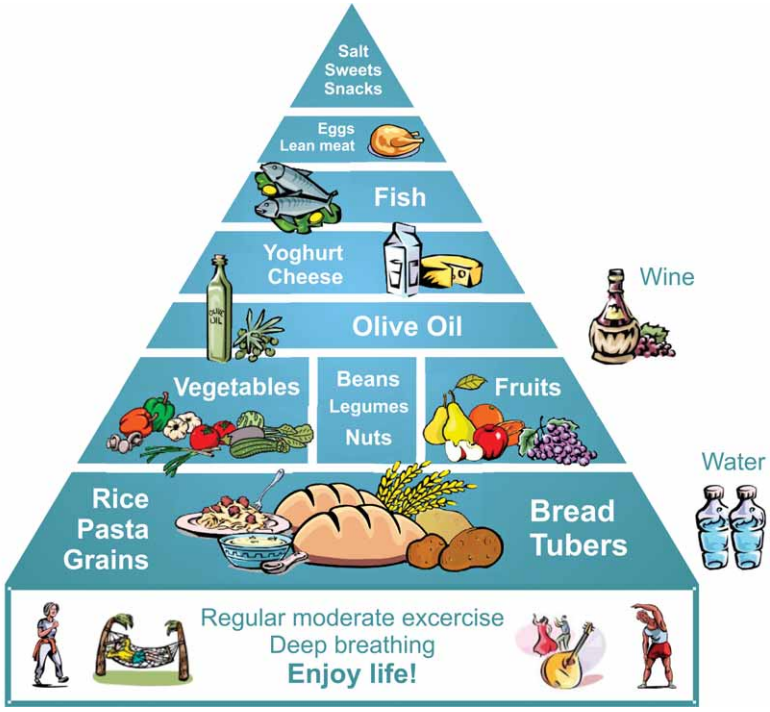

Figure 1. *The new »Holistic Food Pyramid«, optimized according to the basic principles of the Mediterranean diet, our metabolic needs and cellular physiology.*

Stress also plays a part in adherence to a healthy diet in that excessive stress generally implies less time for self-care. Excessive daily demands may result in less time for, and focus on, food purchasing and preparation, thereby leading to increased reliance on convenience foods *(12)*. Stress resulting from a demanding daily schedule may also require that meals are consumed as quickly as possible, contrary to the classic advice for weight-management that slower eating allows satiety to register before too much food is consumed *(46)*.

The »*When* of eating« must fit into a daily schedule that includes adequate rest and exercise. The generally accepted necessity of diet and exercise to be considered concurrently is reflected in the modification of most modern food pyramids to include exercise *(1)*, a life-style pattern which is essential for the future in that physical activity, like adherence to a Mediterranean diet, is associated with decreased obesity in children *(47)*.

Sleep has emerged as an additional life-style factor to consider in healthy diet design wherein more sleep appears to help weight maintenance. Short sleepers, those receiving less than seven hours nightly, have been shown to have 15% higher serum levels of ghrelin, a peptide which stimulates appetite, and 16% lower levels of leptin, a hormone involved in increasing feelings of satiety *(12)*. The lack of leptin may lead to a state of over-nutrition, which is associated with accumulation of triglycerides in non-fat cells. Such fatty deposits cause lipotoxicity, which is associated in the development of diseases such as type 2 diabetes, cardiomyopathy, and insulin resistance *(10)*.

In summary, the spectrum of behavioural and nutritional strategies that help to control appetite and energy intake work cumulatively to tip the balance of the internal milieu from disease-prone toward healthy.

KITCHEN: UTILIZATION IN SPECIFIC CONDITIONS

Inflammation

Inflammation is considered to be a key etiologic factor for the development of illness and is common to metabolic, neoplastic and cardiovascular diseases. The appropriate choice of nutritional components, such as those found in the Mediterranean diet (Figure 1), provides a non-pharmacologic means of preventing and decreasing inflammation. These outcomes are generally measured by improvements in markers such as c-reactive protein (CRP), fibrinogen, homocysteine and LDL *(45)*, changes that are considered substantial health benefits irrespective of weight or weight loss *(48)*. All the same, a healthy BMI provides health benefits that are in part explained by the inverse association between visceral fat mass and anti-inflammatory adiponectin *(49)*.

Obesity

Although too often regarded in the public eye as merely an esthetic concern, obesity is a nutritional disorder that can only be considered as an epidemic with devastating health implications. Currently, the number of people worldwide who are obese is equal to the number who are starving. In particular, most of the population of the

United States will be obese by the late 2040's, according to the current trajectory *(50)*.

Obesity is also an issue in Mediterranean countries, in particular amongst children *(47)*. This is attributable to an increasingly sedentary lifestyle, as well as excess sugary and salty snacks. In our era of rampant globalization, geographic distribution is no longer a key determinant of dietary choices and it is, in fact, the availability of Western-style cheap foods and large chain stores that are taking their toll on dietary choices *(51)*.

Obesity is now understood to be a chronic inflammatory disease *(9)* that is largely influenced by the metabolic activity of adipose tissue *(52)*. In order to make best use of the anti-inflammatory effects of the Mediterranean diet, we must reduce the pro-inflammatory impact of excess adipose tissue. Simple calorie reduction, like red wine, activates SIRT-1 whose health benefits are additive to those gained by this same measure leading to reduced adipocyte mass. Furthermore, increased inflammation in the adipose tissue appears to be, in part, promoted by elevated levels of bacterial-associated lipopolysaccharide (LPS) in the gut *(10, 53)*, thus furthering the impetus to explore the microbiota for a means of intervention in this metabolic disorder.

Glycemic Control

Monounsaturated fatty acids, such as those found in olive oils, are associated with a protective effect on cell insulin sensitivity and viability *(54)*. This may largely be the result of olive oil's effect on thromboxane B (TXB). This parameter is generally associated with and found elevated in diabetes and is lowered after only four days of administering olive oil *(24)*.

As blood glucose levels are adversely elevated by inflammation *(52)*, the Mediterranean diet, with its abundance of olive oil, provides a solid foundation on which to personalize an anti-inflammatory diet, particularly for those in need of glycemic control. The microbiome, a potential target for modification, impacts insulin levels *(10)* as does physical activity *(54)* thus reminding us that the health benefits of a well-chosen diet are incomplete without considering life-style choices as well.

Cancer

As a whole, adherence to the Mediterranean diet reduces the risk of some cancers *(55)* including breast cancer *(56)*. Olive oil in particular has been associated epidemiologically with the reduction of or no increased risk for the development of certain cancers, a factor that is now attributable to olive squalene *(23, 25)*.

Cardiovascular

The risk profile for cardiovascular disease (CVD) has been shown to improve with utilization of a Mediterranean-style diet *(45)*. In the development of atherosclerosis, inflammation serves an integral role and as such, interleukin-6 (IL-6), CRP and adiponectin are indicators of coronary heart disease (CHD) activity *(24, 57)*. Stable CHD patients who supplement their diets with virgin olive oil have been shown to have decreased levels of pro-inflammatory IL-6 and CRP *(24)*. Additionally, anti-inflammatory adiponectin levels are increased with adherence to Mediterranean diet *(57)*.

The potential influence of the Mediterranean diet on cardiovascular parameters being mediated through the metagenome remains a largely unexplored area of research. What has been demonstrated is that myocardial substrate utilization is influenced by host-microbiome interactions *(10)*. Additionally, hypertension, another leading contributor to the development of cardiovascular disease, is a parameter that may be impacted by the metabiome *(10)*. Although dietary intake of polyphenols has been shown to impact the microbiome *(58)*, biophenol-rich olive oil and other components of the Mediterranean diet remain to be investigated with regard to their metagenomic effects. There appears, however, to remain a largely unexplored area into metagenomic targets of nutritional interventions for inflammation-mediated diseases, a subject ripe with potential future preventive health indications *(59)*.

CONCLUSION

Although the search for deep understanding of the nature of the connections between dietary constituents and health outcomes can begin to appear to resemble a quest for Shangri La, the indisputable epidemic of obesity, a pro-inflammatory state, serves as one omnipresent reminder of the need for deeper understanding of readily-available nutritional components which may help tip the scales back toward a healthier future. In order to move forward with a clear perspective of the utilization of the Mediterranean diet, several questions remain to be answered, such as, are there additional health benefits of olive oil which have not yet been explored? To what extent are the anti-inflammatory biophenols of extra-virgin olive oil absorbed? In what ways are these effects mitigated by the microbiome? How does the prevalence of specific gut microbes change according to specific dietary intake? Which dietary substances could provide the most favorable metagenomic phenotypes?

With such a banquet feast of connections to be explored, we can consider the Mediterranean diet as a well-made first course. Similar to the ancient Greek recipe for a long, happy life, *Pan Metron Ariston* (»all good things in moderation«), food, rest and exercise, in balance, remain central to taking self-responsibility for minimizing the risks of disease development. Future investigations can rely on the specific insights offered from studies of the Mediterranean diet as one of many options for orienting further study into the best advice for healthy life-style shifts.

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