14 Some Aspects of the Mediterranean Diet

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CONTENTS

14.1 INTRODUCTION

The Mediterranean region spans 3 continents and more than 15 countries connected by blue seas and magnificent terrain. The Mediterranean area is a world of its own and the Mediterranean lifestyle is a mix of cultures and cuisines. Recent medical research has shown that this cuisine is much better for our health than the typical American diet. While the preparation and seasonings vary from country to country, the people of the Mediterranean region all eat larger amounts of vegetables, fruits, beans, and grains; use olive oil as the main source of fat; and consume moderate amounts of protein, primarily from fish and poultry. By embracing the new trend towards healthier eating, we need to adopt the types of cooking which were developed several decades ago around the Mediterranean Sea.

People have become aware that good health is linked to good diet. The real difficulty has been defining a healthy diet. As we have begun to understand that a healthy diet leads to a healthy lifestyle, the concept of the Mediterranean diet is growing in popularity. The Mediterranean diet, as a model of a healthful diet, has attracted considerable attention because of the low rates of chronic diseases and the high life expectancy noted in countries bordering the Mediterranean Sea. Mediterranean diets are found not only to produce favorable effects on blood lipid profiles,[1] but also protect against oxidative stress and carcinogenesis.[2] The high intake of

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antioxidants and fiber helps to scavenge even the small amount of oxidants or oxidized compounds.[3] Epidemiological studies[4-7] show that Mediterranean populations who get more than 30% of their daily calories from fat and rely on olive oil as their principal source of fat have lower rates of cancer, stroke, and heart disease than Americans on a low-fat diet. In addition to the benefits of olive oil, there are many biologically plausible reasons why the increase in consumption of fruits and vegetables found in the Mediterranean diet might slow or prevent the appearance of cancer. These reasons include, but are not limited to, the presence of such readily bioavailable anticarcinogenic agents as vitamins C and E, carotenoids, fiber, selenium, plant sterols, phenols, and *d*-limonene. A recently published study[8] has demonstrated that the high fish, fruit, and vegetable diet characteristic of Mediterranean countries is helpful in preventing heart disease and subsequent heart attack and stroke, and that switching to this diet also protects people who have already had one heart attack from having another.

14.2 MEDITERRANEAN DIET

As well as being the cradle of civilization, the Mediterranean region is also the source of many of the world's delicious cuisines. The term Mediterranean diet was first used by Ancel Keys, an American physiologist, in his book *How to Eat Well and Stay Well: the Mediterranean Way*.[9] In ancient times, the residents of the Italian peninsula learned most of what they knew about food and nutrition from the Greeks who colonized Sicily and the southern part of Italy. In Sicily and the southern provinces, the original Mediterranean diet is still very much the standard with its emphasis on fruits, vegetables, and pasta.[10]

"Mediterranean diet" is an imprecise term because dietary patterns within the various Mediterranean countries vary widely in consumption of specific foods and in nutrient intake.[11] However, a common Mediterranean diet is characterized by abundant plant foods (fruits, vegetables, cereals, bread, beans, nuts, and potatoes), olive oil as the principal source of fat, dairy products, fish, poultry, and meat in low to moderate amounts, fresh fruit as the typical daily dessert, and use of garlic, onions, lemon or lime, and herbs as condiments.[12] This diet, when consumed in sufficient amounts, provides all of the known essential micronutrients, and other plant food substances believed to promote health. Minimal processing, seasonal use, freshness of foods, as well as methods of preparation would be expected to maximize contents of dietary fiber, antioxidants, other micronutrients, and nonnutritive substances found in plant foods.

There is no single typical Mediterranean diet. Defining and understanding the Mediterranean diet is not easy because there are several countries that border the Mediterranean Sea. Although dietary patterns in Mediterranean countries vary widely in consumption of specific foods and in nutrient intake, both European and non-European Mediterranean populations benefit from the protective effect of the so-called Mediterranean diet on health.[4] The abundant fruits, vegetables, and whole grains, and the low to moderate intake of dairy products in traditional Mediterranean diets are likely to have contributed to the low rates of chronic diseases observed in these populations.[13] The Mediterranean diet is rapidly changing, especially in large

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cities where people are slowly abandoning their traditional diet for a Western-style diet. However, the traditional Mediterranean diet is still consumed in the rural areas of most of the Mediterranean countries.

After thousands of years of defining itself, the Mediterranean cuisine is integrating into our American culture, and for good reasons. Food is fresh and it is high in soluble fiber, antioxidants, and other important nutrient and nonnutrients components. The preference for fresh fruits and vegetables in Mediterranean diets results in a higher consumption of raw plants, a lower production of cooking-related oxidants, and a consequent decreased waste of nutritional and endogenous antioxidants.[3] From the nutritional point of view, this diet is rich in vitamins, minerals, fiber, and minor organic, nonnutritive substances. In addition to the nutrients that are involved in normal metabolic activity, fruits and vegetables contain components that may provide additional health benefits. These food components (generally referred to as phytochemicals) are derived from naturally occurring ingredients and are actively being investigated for their health-promoting potential. The Mediterranean diet is notably low in red meat and saturated fats. Red meat is listed at the top of the Mediterranean diet pyramid,[14] while cheese and yogurt, along with poultry and fish, are the predominant sources of protein. As a result, the Mediterranean diet is low in saturated fat and high in monounsaturated fat, a fact that in part may explain the lower rates of heart disease seen in this part of the world.

14.2.1 Fruits and Vegetables in the Mediterranean Diet

Fresh fruits and vegetables feature strongly in the Mediterranean cuisine. Fresh fruit is often the dessert and is eaten raw. Vegetables are generally eaten raw as a side dish or used for the preparation of rice and pasta dishes, sauces, and vegetable soups. The same vegetable can be prepared with different herbs and spices. The Seven Countries Study[15] probably represents the major investigation contributing to knowledge about the relationship between the Mediterranean diet and coronary heart disease. Kromhout et al.,[16] compared the data of the five Mediterranean cohorts (Italy, Yugoslavia, Corfu, Crete, and Greece) to the three Northern European cohorts (The Netherlands, Eastern and Western Finland) for fruit and vegetable intake. The five Mediterranean cohorts consumed more fruits (222 g/day vs. 51 g/day), more vegetables and legumes (226 g/day vs. 150 g/day), and less pastries and sweets (28 g/day vs. 119 g/day).

Despite relatively high tobacco consumption, lung cancer rates are lower than expected in the Mediterranean region. This may be related to the high intakes of fruits and vegetables.[17] The Mediterranean diet is rich in fruits and vegetables, therefore, it contains a remarkable amount of vitamins. Vitamin C is supplied primarily by consumption of raw fruits, especially citrus fruits, and vegetables. Vegetables such as tomatoes, potatoes, carrots, broccoli, cabbage, and green leafy vegetables, supply vitamin E to the diet. β-Carotene is found predominantly in vegetables such as the carrot, pumpkin, cabbage, and spinach, and in some fruits, especially oranges and apricots. Cooked tomato supplies the diet with notably high quantities of lycopene. Fruit and vegetables are also important sources of fiber that contains mostly pectin and cellulose. A daily intake of 20 g of insoluble fiber from

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fruits and vegetables[18] plus 15 g from other sources, helps regulate both the metabolism of fat and the intestinal function.

14.2.2 Main Characteristics of the Mediterranean Diet

It is difficult to imagine the Mediterranean cuisine without lemons. Lemons, like oranges, are a quintessential part of Italy and the Mediterranean cuisine. In Ancient Rome and Egypt, stories of the lemon's miraculous power against poison persisted.[10]

The main components of a traditional Mediterranean diet[14] are

- An abundance of plant foods, including fruits and vegetables, potatoes, breads and grains, beans, nuts, and seeds, with emphasis on fresh and minimally processed foods
- Total fat ranging from 25% to 35% of energy, with saturated fat no more than 8% of energy intake
- Olive oil as the principal source of fat
- Fresh fruit representing the classic final course of every meal and often is the dessert
- Daily consumption of low to moderate amounts of cheese and yogurt
- Weekly consumption of low to moderate amounts of fish and poultry
- Red meat and sweets are consumed only a few times per month

14.2.3 Food Pyramids

The Food Guide Pyramid has replaced the basic four food groups as the standard for setting up a healthy diet. The USDA pyramid model retains the idea of food groups, but its shape provides the key for interpreting how to make healthy food choices on a daily basis. The broad base highlight the food groups that should constitute the majority of our daily intake, while the top food group of the pyramid should be consumed as little as possible.

The Mediterranean Diet Pyramid is based on relative frequencies and is therefore intended to provide an overall impression of healthy food choices rather than to specify the number and size of recommended servings of certain foods or proportions of energy obtained from them. In the USDA Pyramid animal and plant protein are presented in the same category with a recommended consumption of two to three servings per day, and all fats and oils are combined together at the top of the pyramid. On the other hand, the Mediterranean Diet Pyramid differentiates between fish, poultry, and red meat intake. Fish and poultry are consumed a few times per week while red meat is consumed a few times per month (Figure 14.1).

14.3 MEDITERRANEAN DIET AND CITRUS CONSUMPTION

From 1950 to 1965 the overall fruit and vegetable consumption increased in Italy. The levels reached at that time were then maintained in the following years. The consumption of fresh fruits and vegetables doubled, while the consumption of citrus

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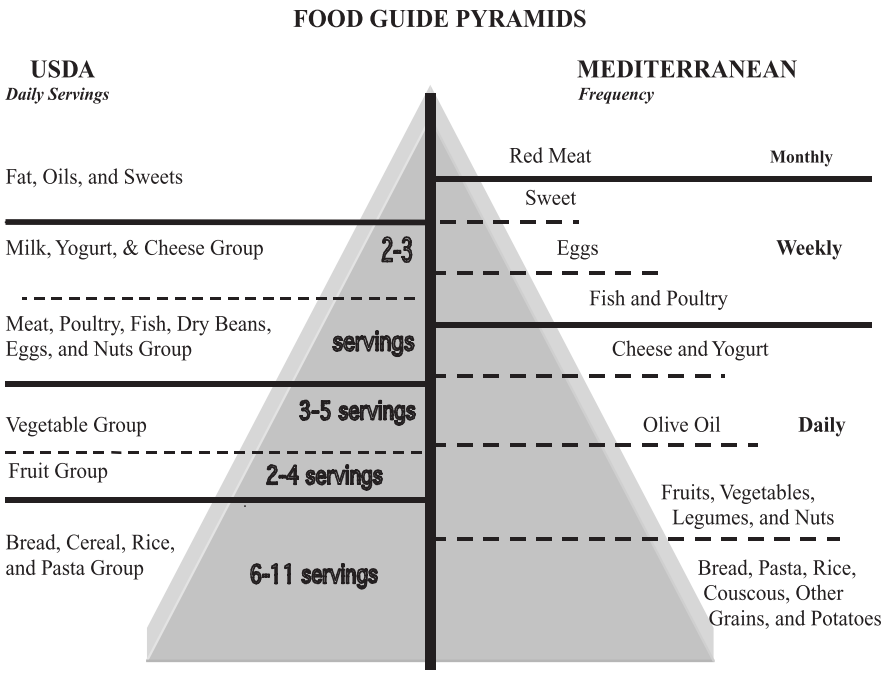

FIGURE 14.1 Food Guide Pyramids.

fruits and tomatoes more than doubled.[19] Citrus fruit is known for its high content of vitamin C, potassium, and *d*-limonene. Vitamin C is an antioxidant that may protect cell membranes and DNA from oxidative damage. Its role in the synthesis of the collagen is crucial; vitamin C deficiency may, therefore, affect the integrity of intercellular matrixes thus promoting tumor growth or hindering tumor encapsulation.[20] Furthermore, there is growing evidence that high-potassium diets may be beneficial in both animal and human genetic hypertension.[21] Citrus fruit also contains *d*-limonene (found specifically in citrus fruit oils), which has been shown to have pronounced chemopreventive and chemotherapeutic activities. Vitamin C and *d*-limonene may contribute to the favorable effects attributable to diets relatively rich in fruits and vegetables, and are associated with a lower incidence of breast, lung, pancreas, colon, rectum, and cervical cancers.

Current amounts of naturally occurring *d*-limonene contained in foods, in portions commonly consumed by the American population, may be inadequate to achieve optimal health benefits. *d*-Limonene is probably present in relatively high levels in the Mediterranean diet compared to U.S. diets, and may be an important component in the putative cancer preventive effect of the Mediterranean diet. The Mediterranean diet includes frequent exposure to citrus oils. Food preparation may play an important role in determining the amount of limonene consumed, since orange zest or parts of the whole lemon fruit are often included during cooking to add a desirable sour taste to the food. Lemon juice (a beverage similar to lemonade)

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is usually prepared by blending the whole fruit, and thus contains more limonene than American lemonade.

Observational epidemiological data obtained from case-control, cohort, or cross-cultural studies have consistently suggested that those living in the Mediterranean area, who consume large amounts of fruits and vegetables in general, and citrus fruits in particular, have the lowest incidence rates for most tumors associated with diet. Thus, the role of *d*-limonene, a nonnutrient compound of citrus fruit oils, merits careful evaluation as an inhibitor of carcinogenesis. Although dietary factors alone may not fully explain the health of Mediterranean populations, available evidence strongly suggests important contributions from the high consumption of fruits, vegetables, and whole grain. Studies of the Mediterranean diet are of particular interest to better understand and quantify this effect in view of the methods of preparation, frequency, and range of fruit and vegetable consumption by these populations.

14.4 LEVELS OF *d-LIMONENE IN CITRUS*

d-Limonene, a monocyclic monoterpene, is a natural component of a variety of foods and beverages and is found in many fruits (especially citrus fruits), vegetables, meats, spices, and other food items.[22] The principal sources of *d*-limonene are the oils of orange, grapefruit, and lemon.[23,24] It is found naturally in orange juice at an average concentration of 100 ppm. The major use of *d*-limonene is as a lemon fragrance in soap and perfume and as flavoring agent in foods, beverages, and chewing gum. *d*-Limonene (orange oil/essence oil) is used as a flavoring ingredient to impart a citrus flavor in artificial oils and can be found in nonalcoholic beverages (31 ppm), ice cream and ices (68 ppm), candy (49 ppm), baked goods (120 ppm), and gelatins, and puddings (48 to 400 ppm).[25] When citrus juices are packed aseptically into laminated cartons, the *d*-limonene content of the juice is reduced by about 25% within 14 days of storage owing to absorption by the polyethylene.[26] Limonene has been found in packaging material at 25 ppm (25 μg/g).[27] Oxidation is not reported to occur to a significant extent in over four weeks of storage in glass.[28] The effects of packaging materials on *d*-limonene contents in commercially available citrus juices has been clearly demonstrated.[28-31] Our data show that the level of *d*-limonene in orange juice samples varied considerably depending on whether the juice is packaged with or without pulp (8 to 10% less in *d*-limonene content). By comparing orange juice samples in glass and plastic containers, we found a 50 to 58% decrease in *d*-limonene levels based on the type of packaging alone.[29]

Daily U.S. per capita consumption of *d*-limonene, as a result of both its natural occurrence in food and of its presence as a flavoring agent, was estimated to be 0.27 mg/kg body weight per day, or 16.2 mg/day for a 60-kg individual.[32] Intake of *d*-limonene can vary considerably, however, depending on the types of food consumed. Citrus juice products are among the richest sources of *d*-limonene: daily intake owing to consumption of these products may approach 1 mg/kg body weight for adults and 2 mg/kg body weight for young children. Preliminary results from our studies in Arizona suggest, however, that source, preparation practices, and packaging materials have major influences on actual *d*-limonene levels in citrus

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products. The largest sources of *d*-limonene (averaged concentrations) identified to date are fresh Mediterranean-style lemonade 600 mg/l and limeade 350 mg/l, followed by commercial orange juices. Many commonly produced citrus beverages actually contain no *d*-limonene.

Human exposure to *d*-limonene through the diet or environment is widespread. However, in human populations, the amount of *d*-limonene ingested is determined not only by the frequency and amount of citrus food intake but also by the amount of citrus oil (peel) consumed.[29] Discussions with individual Americans suggest that peel (zest) consumption is not uncommon, and may vary between ethnic groups. Our current work in Arizona suggests that adults with the highest citrus juice intake (two cups per day) were consuming 20 to 50 mg *d*-limonene per day. In the same study, peel consumption was not uncommon, with one-third of the subjects interviewed (163/470) reporting some citrus peel use. Individuals reporting citrus peel use were consuming 90 to 250 mg *d*-limonene per day.

14.5 METABOLISM OF *d*-LIMONENE

In human subjects and several other mammalian species, oral *d*-limonene is completely absorbed and undergoes extensive biotransformation to active metabolites.[33-35] *d*-Limonene metabolites were analyzed in plasma extracts by gas chromatography. Rapid conversion of *d*-limonene into its two major metabolites, perillic acid and dihydroperillic acid, was detected. These metabolites comprised more than 89% of the circulating limonene-derived material at 1 h after administration and thereafter, whereas *d*-limonene itself accounted for only 15%.[36] The most recent human study[37] has shown humans produce all of the same rat plasma metabolites in approximately the same proportions, and the investigators were able to identify two more metabolites in plasma extracts, namely limonene-8,9-diol and an isomer of perillic acid. A comparison of the rat metabolism of orange oil (95% *d*-limonene, from Sunkist) vs. 99% *d*-limonene (Aldrish) indicated that identical metabolites were formed at identical concentrations.[35] In both plasma[36] and urine, [38] limonene is only a minor component of the total limonene-derived material. Metabolites of limonene have been shown to be more potent pharmacological agents than limonene itself in several experimental systems.[39]

Limonene and/or its metabolites distribute throughout the body, showing some preference for fatty tissues.[36,40] No studies have been done to assess the level of *d*-limonene and its metabolites in fatty tissues. In Japan, limonene has been used locally for the purpose of dissolving gallstones in human gallbladders at daily doses of up to 20 g per patient, or approximately 285 mg/kg, with no overt toxicity.[41] It is extensively metabolized by rats, humans, and other species.

14.6 LIMONENE AND TUMOR REGRESSION

The Mediterranean diet was found not only to produce favorable effects on blood lipid profiles, but also found to protect against oxidative stress and carcinogenesis.[14] The chemopreventive efficacy of limonene during both the initiation and promotion stages of carcinogenesis has been demonstrated in chemically induced rodent mam-

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mary,[42-44] skin,[45,46] liver,[47] lung and forestomach,[48,49] colon,[50] and gastric[51] tumor model systems. *d*-Limonene inhibits activation of the tobacco-specific carcinogen NNK, and accordingly may have the capacity to diminish carcinogenic response to exposures to tobacco.[52]

It was recently demonstrated that dietary *d*-limonene exhibits therapeutic effects against chemically induced mammary tumors in rats, with a regression of >80% of carcinomas with little host toxicity. Chander et al.[53] found that a 10% limonene dose mixed in the diet caused tumor regression in all animals, while a 5% limonene dose was only able to cause regression in 50% of the rats. The limonene dose-tumor regression response relationship is steep. Significant regressions are not observed at 5% dietary levels, while a majority of tumors completely regress above the 7.5% dietary level. Limonene appears to act in a cytostatic fashion. Its removal from the diet results in a significant number of tumor recurrences.[54] Regressing tumors have a unique histopathological appearance that is not associated with gross cytotoxicity, immune cell involvement, or apoptosis. The postinitiation chemopreventive/tumor suppressive activity may be due in part to the inhibition of isoprenylation of cell growth-associated small G proteins such as p21 ras by limonene and its metabolites.[54,55] Posttranslational isoprenylation is required for functionality of these proteins, e.g., transformation by ras.[56,57] Perillic acid and dihydroperillic acid, the two major metabolites of limonene in the rat and human, are more potent inhibitors of small G-protein isoprenylation than is limonene. Perillic acid is a more potent inhibitor of cell growth than is limonene. These findings coupled with the extensive metabolism of limonene *in vivo*[36,38] raise the possibility that the antitumor effects of limonene *in vivo* may be mediated via perillic acid, and perhaps other metabolites.[36] Since farnesylation of ras protein is critical for its ability to cause oncogenic transformation, inhibition of protein prenylation may be the basis of the antitumor effects of limonene and perillic acid.

A minor metabolite of limonene, perillic acid methyl ester, is a potent inhibitor of the enzyme protein farnesyl transferase. These data suggest that if the inhibition of protein prenylation is a mechanism for limonene's anti-cancer activities, this monoterpene may be a prodrug that is converted into pharmacologically active substances by metabolic modification.[58] *d*-Limonene can also inhibit carcinogen-induced neoplasia when administered at a short time interval prior to carcinogen challenge.[44] The initiation-phase chemopreventive effects of *d*-limonene have been attributed to the induction of phase I[59] and phase II[60] carcinogen-metabolizing enzymes, resulting in carcinogen detoxification.[59]

d-Limonene was recently tested in phase I therapeutic trials.[37,61] A single-center phase I clinical trial was undertaken to determine the toxicity and maximum tolerated dose of orally administered *d*-limonene in patients with advanced solid tumors.[37] One partial response in a breast cancer patient on 8 g/m^2 per day was maintained for 11 months and 3 patients with colorectal carcinoma had prolonged stable disease.

A conceptual limitation that has plagued epidemiological studies has been the approach to analysis. In most studies, food intake data have been converted to summarized nutrients, which have been the focus of the risk analysis. Thus, the

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limitations of food composition databases have placed important contraints on what could be analyzed. In addition, in studies that have analyzed food intake by food groups as well as by nutrients, food groups have generally been found to have greater relative risks (or relative protection) associated with them than specific nutrients. This may be due both to the presence of important compounds not included in standard food composition databases and to the importance of combinations of factors present in whole foods. The Arizona population offered a unique opportunity to study potential associations between citrus consumption and risk of skin squamous cell carcinoma (SCC). A total of 274 men and 196 women who had participated in the Southeastern Arizona Skin Cancer Study completed a citrus consumption study. Our results show that peel consumption, the major source of dietary *d*-limonene, was not uncommon and may have a potential protective effect in relation to skin SCC. We found no association between the overall consumption of citrus fruits or citrus juices and skin SCC. However, the most striking feature was the protection purported by citrus peel consumption. Moreover, there was a dose-response relationship between higher citrus peel in the diet and degree of risk lowering.[62]

14.7 CONCLUSION

The benefits of phytochemicals have been extensively publicized in the popular press, which has resulted in increased public awareness and interest in consumption of phytochemical-rich foods as a method of enhancing health and well-being. Health benefits of these foods are best obtained through the consumption of a varied diet using our normal food supply. The Mediterranean cuisine is able to offer hundreds of delicious and nutritious recipes and is easily accepted by most people. Therefore, the Mediterranean diet provides the best chance for influencing people to abandon unhealthy foods in favor of fresh vegetables, fruits, grain, and olive oil. It introduces a dietary pattern that is attractive for its delicious palatability as well as for its health benefits. Of course, food alone is not the answer. Mediterranean area inhabitants were and, in many regions, still are more physically active than Americans.

Data from several clinical trials,[63-66] indicate that the beneficial effects associated with a diet high in fruits and vegetables may not be demonstrated when individual nutrients, such as vitamins C and E or beta-carotene, are consumed in supplement form. Neverless, pharmaceutical companies will be motivated to isolate components in foods into pill or supplement form to market the individual components for their health benefit(s). We don't have to wait for more analyses of individual components of the diet when it is clear that the whole diet is important. The public must be convinced that the more appropriate choice would be to increase fruit and vegetable consumption and choose a varied diet based on the principles of the Mediterranean Food Guide Pyramid. However, whether or not the Mediterranean diet is followed, always include regular exercise as a part of a healthy lifestyle. It has become increasingly clear that the Mediterranean Diet is one of the best ways to maintain lifetime wellness and prevention of chronic diseases.

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