The Mediterranean diet: Effects on proteins that mediate fatty acid metabolism in the colon

Zora Djuric
Departments of Family Medicine and Environmental Health Sciences (Nutrition Program), University of Michigan, Ann Arbor, Michigan, USA

Abstract
A Mediterranean diet appears to have health benefits in many domains of human health, mediated perhaps by its anti-inflammatory effects. Metabolism of fatty acids and subsequent eicosanoid production is a key mechanism by which a Mediterranean diet can exert anti-inflammatory effects. Both dietary fatty acids and fatty acid metabolism determine fatty acid availability for cyclooxygenase- and lipoxygenase-dependent production of eicosanoids, namely prostaglandins and leukotrienes. In dietary intervention studies and in observational studies of the Mediterranean diet, blood levels of fatty acids do reflect dietary intakes but are attenuated. Small differences in fatty acid levels, however, appear to be important, especially when exposures occur over long periods of time. This review summarizes how fat intakes from a Greek-style Mediterranean diet can be expected to affect fatty acid metabolizing proteins, with an emphasis on the metabolic pathways that lead to the formation of proinflammatory eicosanoids. The proteins involved in these pathways are ripe for investigation using proteomic approaches and may be targets for colon cancer prevention.

Keywords
dietary intervention; fatty acids; inflammation; Mediterranean diet; proteomics

INTRODUCTION
There is substantial epidemiological evidence that indicates dietary patterns influence colorectal cancer risk.1–3 One such dietary pattern that holds great promise for cancer prevention is the Mediterranean dietary pattern, which is based on the Greek diet consumed in Crete. All of the major components of the traditional Cretan diet, namely olive oil, fish, cereals, legumes, fruits, and vegetables, have been associated with decreased colorectal cancer risk.4–10 Relative to the American diet, this diet results in lower intake of n-6 versus n-3 and n-9 fatty acids, lower intake of polyunsaturated fatty acids (PUFAs), lower intake of red meat, and much higher intake of plant-based foods and monounsaturated fatty acids (MUFAs).11–14

In intervention studies and in observational studies of the Mediterranean diet, blood levels of fatty acids do reflect dietary intakes, but the differences in blood levels among populations are much smaller than the differences in dietary intakes (Table 1). It is, therefore, very
important to consider the absorption, distribution, and metabolism of fatty acids since these factors will limit changes in blood and tissues when the diet is changed. Small differences in fatty acids may, nonetheless, be important, especially when exposures occur over longer periods of time, and may be responsible for the preventive effects of a Mediterranean diet against colon cancer. This review evaluates the potential impact of this type of eating pattern on fatty acid metabolic pathways. Fatty acid metabolism regulates the amount of arachidonic acid that is available in cell membranes for subsequent formation of proinflammatory eicosanoids. Eicosanoids, particularly prostaglandin E\(_2\) (PGE\(_2\)), have been shown to be key mediators of colonic carcinogenesis.\(^{15}\)

**MEDITERRANEAN DIET AND COLON CANCER RISK**

Every person in the industrialized world has, on average, a 1 in 20 chance of developing colorectal cancer in his or her lifetime, and rates in the United States are the highest (55 cases/100,000 population in 2000).\(^{1, 16, 17}\) Dietary practices have been implicated in the risk of colorectal cancer, although dietary effects per se may be difficult to disentangle from obesity effects. Risk is typically lower in population groups that do not follow the typical American diet, and risk has been shown to increase in migrants who move to the United States.\(^{17}\) In Greece, rates of colorectal cancer are very low, but they are elevated in Greeks who have moved to Australia.\(^{18-20}\) Rates of colorectal cancer mortality in Greece in 1955 were 5–8/100,000 population and have increased to about 10/100,000 in 2000, perhaps as diets have drifted away from the traditional patterns.\(^{21}\)

Case-control studies of colorectal cancer risk and diet have fairly consistently found that intakes of fruit, vegetables, fish, and whole grains are protective, while red meat and refined carbohydrates are associated with increased risk.\(^{4, 5}\) In the Breast Cancer Demonstration Project, increased compliance with a Recommended Food Score was associated with a 51% reduction in colorectal cancer risk.\(^{22}\) However, increased fruit and vegetable consumption alone, from a pooled analysis of 14 studies, reduced only distal colon cancer risk.\(^{23}\) Eating patterns may, therefore, play a more important role in colon cancer risk reduction than consumption of any specific food or nutrient, and this may be the ideal approach for prevention.\(^{24-27}\)

A complex mixture of protective compounds can only be obtained from a dietary pattern-based approach, and methods have been developed to score adherence to a Mediterranean-style diet. In persons who followed a Mediterranean eating pattern, recurrence of adenomatous polyps was decreased, with an odds ratio of 0.3 for the third versus the first tertile.\(^{8}\) In one large Greek study, a Mediterranean diet score was constructed to reflect the traditional Mediterranean diet. Higher scores were significantly associated with decreased mortality from all cancers (24% reduction in mortality for every 2-point increase in the score), but generally, intakes of specific foods were either not associated with mortality or the association was much weaker.\(^{28}\) Note that in studies done in the United States, the extent of Mediterranean-style eating, as defined by being above/below median values, will differ from that in European studies due to differences in the median intakes of foods. In two recent US studies, a Mediterranean dietary pattern had a significant protective effect against colon cancer in men only.\(^{29, 30}\) In the American Association of Retired Persons cohort, however, total cancer mortality in both men and women decreased significantly along with increased adherence to a Mediterranean dietary score.\(^{31}\)
MECHANISMS BY WHICH FATTY ACIDS CAN AFFECT COLON CANCER RISK

A unique aspect of the Mediterranean diet is the different type of fat that is ingested. Increasing fiber alone does not appear to be sufficient to significantly impact the risk of colon cancer, and increased intakes of fruit and vegetables has been shown to have modest effects. Fatty acids are substrates for eicosanoid production, and eicosanoids can activate proinflammatory pathways that promote colon carcinogenesis. PGE2, formed from arachidonic acid by constitutive cyclooxygenase 1 (COX-1) and inducible cyclooxygenase 2 (COX-2) in the colonic mucosa, plays an important role in the expansion of cell populations in the colonic crypt and subsequent formation of adenoma. Cyclooxygenase (COX) inhibitors conversely block proliferation, induce apoptosis, and inhibit angiogenesis in the colon. Inhibition of both COX-1 and COX-2 appears to be effective for preventing polyp formation and greatly reduces colon cancer risk. It appears that reducing the level of PGE2 levels in normal tissue could lead to a reduced risk of polyp formation, and PGE2 has been identified as an appropriate prevention endpoint.

There are several examples of eicosanoid modulation by dietary fats. The colon-tumor-promoting effects of corn oil, which is very high in linoleic acid (18:2, n-6), have been related to COX-2 induction, while diets rich in high-fat fish oil have been found to decrease COX-2 protein expression. Intervention with a Mediterranean dietary pattern does not greatly increase n-3 fatty acid intakes in the manner that fish oil supplementation would, but n-6 fatty acid intakes are decreased by 30% or more to help shift the n-3 : n-6 ratio, which greatly impacts the formation of PGE2 and leukotrienes. In rats, the n-3 : n-6 dietary ratio was found to be more important than the total amount of n-3 fatty acid intake in inhibiting 12-hydroxyeicosatetraenoic acid (12-HETE), 6-keto-prostaglandin F2α, and thromboxane B2. The role of n-9 fatty acids, such as oleic acid (18:1) found in olive oil, has not been studied as extensively, but Bartoli et al. observed inhibition of aberrant crypt foci and adenocarcinomas, decreased mucosal arachidonate (20:4), and decreased PGE2 in rats fed either n-9 or n-3 diets relative to rats fed diets high in n-6 fatty acids.

COLONIC PROTEOME AS A MEDIATOR OF DIETARY EFFECTS ON MUCOSAL FATTY ACIDS AND EICOSANOID

Dietary fatty acids can impact both the types and the levels of eicosanoids produced in the colonic mucosa. The proteins of interest that can mediate the effects of dietary fatty acids on colonic fatty acid and eicosanoid levels are depicted in Figure 1. These have been selected for this review based on their possible contribution to altering fatty acid ratios in cells, and many have been shown to be relevant to changes during colon carcinogenesis as well. The link between diet and eicosanoid production has barely been studied, yet the impact of metabolism on limiting changes in membrane fatty acids and, subsequently, eicosanoids is important for determining colon cancer risk.

Fatty acid-binding proteins

Role in fatty acid metabolism—There are a number of proteins involved in binding fatty acids for the purpose of transport between and within cells. Among them, perhaps the best-described are the fatty-acid-binding proteins (FABPs). These proteins are abundant in the cytosol of cells, can bind many hydrophobic ligands, and are induced by increased levels of fatty acids. FABPs bind fatty acids and fatty acyl coenzyme A (CoA) with high affinity in the cytoplasm and then translocate to the nucleus. The nuclear receptors for liver FABPs appear to be peroxisome proliferator-activated receptors (PPARs) alpha and gamma, which in turn regulate production of FABPs and other genes needed for nutrient metabolism.
FABPs from the same tissues across species have similarities in fatty acid binding affinity, while there is wide variation in the affinity of FABPs from different tissues. Two forms of FABPs have commonly been identified in the human colon, namely liver FABP (L-FABP) and intestinal FABP (I-FABP), with levels of L-FABP being higher. In a proteomic study, I-FABP was a major protein identified in human intestinal scrapings from both the small and large intestine using two-dimensional gel electrophoresis, although the I-FABP level was twofold higher in the small versus the large intestine.

I-FABP appears to be involved in absorption of fatty acids from the intestinal lumen and synthesis of triglycerides, while the role of L-FABP may be related more to the uptake of fatty acids from the plasma for energy production and phospholipid synthesis. The mechanism of fatty acid movement has been investigated with a model fatty acid, and it was shown that transfer of fatty acids to L-FABP and I-FABP occurs via different mechanisms, with I-FABP having a bigger role in the transfer of fatty acids between membranes and sites of metabolism and L-FABP having the ability to buffer high levels of fatty acids in enterocytes. The I-FABP in humans has a higher affinity for PUFAs than for oleic acid or saturated fatty acids.

Role in colon cancer risk—Both I-FABPs and L-FABPs have been shown to be decreased in colon carcinoma. Conversely, increased expression of L-FABP in colorectal cancer and metastatic foci was associated with increased survival, pointing to the protective role of L-FABP. L-FABP contains a PPAR response element and can bind two ligands simultaneously, unlike other FABPs, and activation of PPAR gamma can protect the colon against colitis.

In addition to binding fatty acids, FABPs appear to mediate the signaling associated with fatty-acid-induced inflammation in cells. Several FABPs have been shown to stabilize leukotriene A4 in cells, which could then have an impact on inflammatory states and hence colon cancer risk. Work in transgenic mice indicated that deletion of adipocyte FABP did not alter phenotype greatly, but the response to inflammatory stimuli was hampered. Interestingly, levels of epidermal FABP in skin were 50% lower in transgenic mice that overexpress COX-2, an enzyme known to increase during colonic inflammation and tumorigenesis.

Effect of diet—the effect of a high-fiber wheat-bran diet that decreased colonic tumors in rats resulted in increased L-FABP and I-FABP in exfoliated colon cells, which would be consistent with a protective effect of FABPs. Soy or whey protein diets, however, decreased colonic I-FABP in carcinogen-treated rats. Oleic acid, a main component of olive oil, which is one of the staples of Mediterranean diets, increased L-FABP and I-FABP in the small intestine, but linoleic acid was even more stimulatory in rodent models. The effects of a Mediterranean diet on FABPs are therefore difficult to predict.

The effects of diet on FABPs may also be affected by genotype. In humans, a fairly prevalent polymorphism in I-FABP (Ala54Thr) was shown to be important in mediating the effects of diet on insulin resistance. Carriers of the Thr54 allele had higher glucose levels than individuals with the Ala54/Ala54 homozygote, but only the Thr54 carriers displayed decreases in glucose levels with a low-fat or Mediterranean diet. Thr54 carriers also maintained better insulin sensitivity when consuming olive oil diets versus sunflower oil diets, while the type of fat intake did not affect those with the Ala54a/Ala54 homozygote. In obese children who were carriers of the Thr54 allele, activation of delta-6 fatty acid desaturase was impaired in response to low levels of arachidonic acids, indicating the inter-relationships of these pathways.
Fatty acid synthase

Role in fatty acid metabolism—*De novo* synthesis of non-essential fatty acids can markedly contribute to tissue levels. Fatty acid synthase (FASN) is a 250-kDa protein responsible for the *de novo* synthesis of long-chain fatty acids, which are stored as triglycerides and used as a source of energy when needed.\(^74\)

Role in colon cancer risk—FASN is expressed in normal human colon at the base of the crypts and is greatly over-expressed in colon tumors.\(^75,76\) Overexpression of FASN was associated with worse colon cancer survival rates in overweight and obese (but not normal-weight) individuals.\(^77\) In fact, inhibition of FASN has been suggested to be a viable therapeutic option for diabetes and cancer.\(^78\) Inhibition of FASN has been suggested to be a target for cancer prevention.\(^79\) Several dietary chemopreventives (soy, epigallocatechin gallate, and acer) have been shown to inhibit FASN.\(^80–82\)

Effect of diet—Of importance to Mediterranean diets, oleic acid downregulates FASN protein expression in cultured tumor cells, which, in turn, downregulates expression of human epidermal growth factor receptor 2 (HER-2).\(^83\) In human colonic carcinoma cell line 2 (CaCo-2) cells, eicosapentaenoic acid (EPA, also known as 20:5, n-3) inhibited FASN much more strongly than did linoleic acid (18:2, n-6).\(^84\) In breast cancer cells that overexpress FASN, n-3 fatty acids and gamma-linolenic acid, but not other n-6 fatty acids, inhibited FASN activity and, possibly, expression.\(^85\) In glioma cells, oleic acid inhibited fatty acid synthesis.\(^83,86\) In vivo, a high-fat diet induced FASN, and FASN was overexpressed in a high proportion of aberrant crypt foci in human colon.\(^87,88\)

Highly unsaturated fatty acids of 20-carbon length and greater are much more efficient than 18-carbon fatty acids in inhibiting FASN, but a caveat is that most studies have been done in experimental models using very high levels of fatty acids that do not mimic human exposures well.\(^89\) Experimental models, however, do suggest that increased intakes of olive oil might decrease protein levels of FASN.\(^78\) Both olive oil and fish oils, therefore, would be expected to decrease FASN expression.

Desaturases and elongases

Role in fatty acid metabolism—Stearoyl CoA desaturase (SCD-1) catalyzes the conversion of palmitate and stearate to their respective saturated fatty acids palmitoleate (16:1) and oleate (18:1), making it highly relevant to dietary interventions that are high in MUFAs. The other major desaturases are delta-5-desaturase (FADS1) and delta-6-desaturase (FADS2). FADS1 is involved in synthesis of 20:5, and FADS2 is the rate-limiting step in synthesis of both arachidonic acid (also known as 20:4, n-6) and EPA (20:5, n-3) from linoleic acid (18:2, n-6) and linolenic acid (18:3, n-3), respectively. Dietary intakes of arachidonic acid in humans are very low, about 110–180 mg/day for adults (of the 67–90 g/day total fat intake), yet this fatty acid compromises 5–10% of the phospholipid-derived fatty acids.\(^90\) This indicates that substantial conversion of linoleic acid (18:2, n-6) takes place to form arachidonic acid. Analogous pathways exist for the conversion of linolenic acid (18:3, n-3) to EPA (20:5, n-3), but the efficiency of this process is about 10% and it is inhibited by high intake of n-6 fatty acids.\(^91,92\)

Desaturases and elongases (denoted Elovl for “elongation of very-long-chain fatty acids”) are regulated in coordination with each other in the synthesis of long-chain fatty acids.\(^93\) There are seven elongases that have been identified, none of which are specific to the colon, but Elovl 1, 5, and 6 are expressed in many tissues. Elovl5 is relevant to eicosanoid synthesis because it is involved in the conversion of 18:2 (n-6) to 20:4 (n-6) and 18:3 (n-3)
to 20:5 (n-3). In diabetes, increased retinal inflammation has been linked with decreased levels of n-3 fatty acids due to a decrease in expression of elongases.

**Role in colon cancer risk**—Inhibition of FADS2 impeded intestinal tumorigenesis in Min mice. Fatty acid synthesis and lipid droplets, which store triglycerides, are increased in human colon cancer, implicating the importance of fatty acids in carcinogenesis.

**Effect of diet**—A high-PUFA diet suppresses SCD-1, while a high-carbohydrate diet increases SCD-1. The n-3 fatty acids are the preferred substrates for elongation and desaturation relative to n-6 fatty acids, but high n-6 : n-3 ratios inhibit n-3 incorporation into membranes. With a Mediterranean diet, which is high in MUFAs, SCD-1 would likely be decreased when MUFAs are plentiful, while FADS2 may be increased to maintain arachidonate levels. With a low-PUFA diet, FADS2 expression was increased.

Exercise and energy restriction have both been shown to enhance elongation of n-3 fatty acids, resulting in increased levels of 20:5 (n-3) and 22:6 (n-3) in rodent skin without increased dietary intakes. When dietary n-3 fatty acids are increased using a fish-oil diet, expression of Elovl5 and desaturases is induced in rat liver relative to olive-oil diets. The changes observed with a Mediterranean diet will then depend on the levels of n-3 fatty acid intake. Elovl6, which is involved in the endogenous synthesis of MUFAs, will likely be decreased. Interestingly, Elovl6 knockout protected animals from insulin resistance induced by a high-PUFA diet.

**Phospholipase A**

**Role in fatty acid metabolism**—Levels of free fatty acids are very low in cells, and most fatty acids are stored in phospholipids or triglycerides. Phospholipids in membranes appear to provide arachidonic acid (20:4, n-6) for eicosanoid production, with triglycerides replenishing the phospholipid stores. Fatty acids are released from phospholipids by phospholipase A when needed for production of energy or to mount an inflammatory response. Upon stimulation of leukocytes, the enzymes involved in eicosanoid synthesis, including PLA2 and cyclooxygenases, act together in concert.

Three PLA2 forms are thought to be primarily involved in arachidonate release upon stimulation: type IV cytoplasmic (c)PLA2α and type IIA and type V secreted (s)PLA2 enzymes.

**Role in colon cancer risk**—In human colon cancer, cPLA and sPLA type IIA are the most studied, but sPLA type X may also be important. Cytosolic PLA2 is generally regarded as the rate-limiting step in the release of fatty acids for eicosanoid production, and there are three isoforms, with cPLA2α being prototypic. It is not known whether cPLA2α could provide the bulk of arachidonate used in early or late prostaglandin synthesis or whether cPLA2 could simply act as a catalyst to stimulate secretion (or synthesis) of sPLA2 necessary for early or late prostanoid synthesis. sPLA2 may also function as a cytokine to help initiate and potentiate inflammation. In the colon, sPLA2 type IIA has been identified as a cancer susceptibility gene. However, it does appear that the secretory and cytoplasmic PLA2 forms interact with each other to release arachidonic acid, making them all relevant in an examination of factors that can modulate inflammation.

Data on the expression of PLA2 enzymes during carcinogenesis differs between mouse models and humans. In humans, both protein and mRNA of sPLA2 was elevated in five of six colorectal adenomas from persons with familial adenomatous polyposis, and this was associated with increased levels of arachidonic acid and COX-2 in the adenomas. Another group examined human colorectal carcinomas and found that a major percentage of samples strongly expressed sPLA2, but staining for cPLA2 was weaker. A similar scenario was
found in Barrett’s esophagus.\textsuperscript{116} A third study found no change in the expression of sPLA\textsubscript{2} type IIA but increased expression of cPLA\textsubscript{2} and COX-2 in tumors.\textsuperscript{117} Consistent with this, a pro-apoptotic role has been proposed for cPLA\textsubscript{2},\textsuperscript{118} but not all studies have found lower cPLA\textsubscript{2} in colon tumors versus normal tissue.\textsuperscript{109} Increased sPLA\textsubscript{2} type II activity in inflamed mucosa of patients with Crohn’s disease and ulcerative colitis was attributed to increased protein levels, about threefold above that of healthy, control mucosa.\textsuperscript{119} In the rat model, PLA\textsubscript{2} expression was higher in tumors than in normal colonic mucosa.\textsuperscript{120,121}

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Effect of diet—In the rat model, expression of PLA\textsubscript{2} in colonic mucosa was greater with a high-fat diet than with a low-fat diet.\textsuperscript{121} In humans, PLA\textsubscript{2} expression has not been evaluated in the colon relative to dietary change. In plasma, however, lipoprotein PLA\textsubscript{2} levels were decreased with a low-calorie diet but were unaffected by supplementation with n-3 fatty acids.\textsuperscript{125,126} In baboons, lipoprotein PLA\textsubscript{2} activity was increased by a high-fat diet, but there was a significant interaction by genotype.\textsuperscript{127}

Cyclooxygenases and lipoxygenases

Role in fatty acid metabolism—Many eicosanoids are formed from the COX-mediated and lipoxygenase (LOX)-mediated metabolism of the 20-carbon fatty acids that are released from phospholipids by PLA\textsubscript{2}. Eicosanoids, in turn, mediate many important biological functions. The prostanoids produced from COX tend to be best known for their involvement in reproduction and inflammation. Thromboxanes are also derived from COX and stimulate platelet aggregation, balancing the effects of prostanoids. Leukotrienes and hydroxyeicosatetraenoic acids (HETEs) produced from LOX-mediated metabolism of arachidonic acid have important roles in vascular tone, renal function, and pulmonary function, e.g., allergic reactions.

Role in colon cancer risk—PGE\textsubscript{2} is the eicosanoid that has been the most widely studied with regard to colon cancer risk. PGE\textsubscript{2} is formed by constitutive COX-1 and inducible COX-2 in the colonic mucosa, and it plays an important role in the expansion of cell populations in the colonic crypt and subsequent adenoma formation.\textsuperscript{35–39} Inhibition of both COX-1 and COX-2 appears effective for preventing polyp formation.\textsuperscript{40} Reducing the level of PGE\textsubscript{2} in normal tissue, therefore, could lead to a reduced risk of polyp formation, and PGE\textsubscript{2} levels have been indicated to be an appropriate candidate for a prevention endpoint.\textsuperscript{43} Interestingly, when EPA (20:5, n-3) is utilized as the substrate by COX, PGE\textsubscript{3} is produced, which has lower proinflammatory action.\textsuperscript{128} Suppression of PPAR delta and PGE\textsubscript{2} with elevation of PGE\textsubscript{3} has been suggested to be the mechanism by which fish oil and pectin enhance colonocyte apoptosis.\textsuperscript{129}

The literature strongly points towards an important role of COX and its metabolites in colon cancer, but the eicosanoid metabolic system is intricately linked and, presumably, regulated as a whole system. The products of 5-LOX and 12-LOX have been implicated in carcinogenesis, while the products of 15-LOX have not. The main products of 5-LOX and 12-LOX that have been studied are leukotriene B\textsubscript{4} (LTB\textsubscript{4}) from 5-LOX and 12-HETE from
Levels of PGE2, LTB4, and 12-HETE were higher in inflamed versus normal mouse mucosa, while 15-HETE, from 15-LOX, was not increased and inhibited inflammation. The two 15-LOX products, 13-S-HODE and 15-HETE, induce apoptosis of colon cancer cells in vitro and can reflect enhanced differentiation of the cells. Accordingly, levels of 15-HETE in serum were lower in colon cancer patients versus controls, and aspirin increased 15-HETE formation. In rats, 12-HETE appears to be involved in stimulating the proliferation of colonic epithelial cells. In Min mice, data indicates a shift from COX-mediated to 12-LOX-mediated metabolism of arachidonic acid in polyps. LTB4 stimulated the proliferation of HT-29 and HCT-15 colon cancer cells in vitro, but leukotriene B5 (LTB5), an isomer of LTB4 derived from metabolism of EPA by 5-LOX, did not. The available data thus identify the COX and LOX enzymes that can be targeted as pro- or anti-carcinogenic.

Effect of diet—Many studies have examined the impact of dietary changes on eicosanoid production. Dietary corn oil and other n-6 fatty acids increased PGE2 levels and colon tumorigenesis, while n-3 and n-9 fatty acids (from fish and olive oil, respectively) have the opposite effect. Most studies found that increased intake of n-3 fatty acids resulted in decreased formation of LTB4, including that in rat colonic mucosa, and increased synthesis of 5-series leukotrienes, as shown in Figure 2. Similarity, dietary eicosatrienoic acid (20:3, n-9) decreased LTB4 synthesis in rat peritoneal cells, and this effect was maximal when n-6 fatty acid intake was low.

Other aspects of the Mediterranean diet, namely the increased consumption of fruit, vegetables, and olive oil, could affect eicosanoid pathways as well. Phytochemicals, which have antioxidant properties, are plentiful in plant foods and would be expected to affect eicosanoids, since oxidative stress induces expression of COX-2. Olive oil contains many phenolic compounds with antioxidant and anti-inflammatory properties as well as oleic acid (18:1, n-9), which suppresses COX-2 via its effects on HER-2/neu receptors. Phenolic compounds in olive oil also were shown to inhibit leukocyte 5-LOX expression, and hydroxytyrosol, also found in olive oil, inhibited formation of LTB4. Plasma levels of LTB4 were decreased in humans consuming extra-virgin olive oil. Olive oil decreased COX-2 protein levels in colon tissue of mice with colitis. Oleocanthal from olive oil was shown to inhibit the expression of both COX-1 and COX-2. Thus, a Mediterranean diet would be expected to decrease COX-1, 5-LOX, and 12-LOX expression and to increase 15-LOX expression in normal mucosa.

Prostaglandin E synthase

Role in colon cancer risk—Although prostaglandin E synthase (PGES) does not metabolize fatty acids directly, it is important to examine it in the context of colon carcinogenesis because of its role in the synthesis of PGE2 from the PGH2 produced by COX. Cytosolic PGES (cPGES) is constitutively expressed and complexes with COX-1. There are two other forms of PGES: microsomal PGES-1 (mPGES), which is induced by proinflammatory stimuli, and membrane-associated PGES-2, which is constitutively expressed and co-localizes with both COX-2 and COX-1. Deletion of mPGES-1 suppressed intestinal carcinogenesis greatly in one Min mouse model but not in another. mPGES-1 was shown to be induced in human inflammatory bowel disease and was overexpressed in 15 of 18 human colorectal cancer samples.

Effect of diet—There are no studies yet available on the effects of dietary fatty acids on PGES levels or activity in the colon. Resveratrol, a compound found in red grapes, attenuated chemically induced colonic inflammation in mice and decreased levels of both...
PGES-1 and COX-2 proteins. In humans, a polymorphism in PGES was important for modulating the effects of fish intake on the risk of colorectal adenoma.

**EFFECTS OF HIGH-MUFA DIETS ON LEVELS OF FATTY ACIDS IN BLOOD**

A number of studies have examined the effects of a diet high in MUFAs on levels of fatty acids in blood (Table 1). These studies have indicated that changes in the levels of fatty acids in blood, especially in the levels of PUFAs, are considerably smaller than the changes in dietary fatty acids. Significant beneficial health effects on insulin sensitivity can nevertheless be achieved, especially if saturated fat or total fat is not too high. Cardiovascular health was the focus of the Lyon Heart Study, and in that study a modified Mediterranean diet supplemented with a high-MUFA spread resulted in changes in fatty acids that were typically in the range of 10–15% (percent difference intervention versus control). This dietary change was sufficient to result in a relative risk of 0.28 for cardiac deaths and a relative risk of 0.39 for cancer deaths relative to the group receiving the American Heart Association Step I diet. Several other interventions with the Mediterranean diet have also resulted in significant benefits with regard to markers of cardiovascular risks, insulin resistance, and incidence of type II diabetes. In fact, many domains of human health have been shown to be affected by Mediterranean diets. This indicates that metabolic pathways limit the impact of diet on fatty acids in blood but that small changes in the levels of fatty acids in blood may be important for health outcomes.

Table 1 shows changes in the levels of fatty acids in blood in several studies that successfully increased MUFA intakes. In a fairly comprehensive exchange-list Mediterranean intervention performed in healthy women, changes in the levels of fatty acids in blood are among the higher increases reported in the literature for self-selected diets (Table 1). In that study, the correlation between the change in dietary MUFA intake and MUFA levels in plasma (from baseline to 6 months) was modest but significant ($r = 0.42, P = 0.001, \text{Spearman}$). Correlations of changes in dietary saturated fatty acids and PUFAs, with their respective blood measures, were much lower, with $r < 0.1$ in each case. In the study of Paniagua et al., in which all food was provided to study participants, changes in the levels of fatty acids in blood were similarly large. Despite the smaller changes observed in the KANWU, Medi-RIVAGE, and PREDIMED studies, significant health benefits were found for diabetes and markers of cardiovascular disease risk and insulin sensitivity.

**EFFECTS OF HIGH-MUFA DIETS ON LEVELS OF FATTY ACIDS LEVELS IN THE COLON**

The relationship between dietary fatty acids and fatty acids in plasma and tissues is fairly well established, with changes in plasma occurring more rapidly than in tissues. The increase in 18:1 in adipose biopsies with a high-MUFA weight-loss diet was 4% over 6 months, which was significant but very small in magnitude. There are, however, examples of large changes in tissues. For example, 6 weeks of a high-PUFA diet resulted in a 36% increase in the level of linoleic acid in cheek cells. Changes in fatty acids in breast adipose tissue biopsies were much greater than in gluteal adipose tissues of women supplemented with 10 g of fish oil per day for 3 months.

Much less data is available on fatty acids in the colon. In persons with ulcerative colitis, 18 g/day olive oil supplementation over 12 weeks significantly decreased 18:0 and 22:6 (30–40%) and significantly increased 18:1 (n-9) by 38% in mucosa. Arachidonic acid (20:4, n-6) was decreased by 40%, but this was not statistically significant. Arachidonic acid has...
been shown to be increased in inflammatory conditions of the colon. Arachidonic acid was also increased in mucosa from persons with inflammatory bowel disease, while the ratio of oleic acid to saturated fatty acids was increased in comparison with normal controls.

Other studies in colonic mucosa have investigated changes with disease progression from normal mucosa to adenoma and cancer. These studies showed that, generally, arachidonic acid increased (but 18:2 decreased, perhaps due to increased metabolism to arachidonic acid), n-3 fatty acids decreased, n-6 : n-3 ratios increased, and MUFAs decreased in comparison with normal controls. This indicates the relevance of these fatty acid COX substrates to colon cancer risk. Fatty acid levels in the normal colonic mucosa, which served as the “control” for the aforementioned studies, are useful in estimating the expected variability in fatty acids between individuals. Typically, the coefficient of variation for arachidonic acid was about 15–20%. One study published an observed range of fatty acid values, which was quite large in normal mucosa from eight healthy individuals (Table 2). The relationships between colonic fatty acid levels and colonic inflammation, however, remain to be determined.

EFFECTS OF DIET ON THE COLONIC PROTEOME

There is precedent for proteomic changes in the colon caused by diet or dietary compounds, and some of those same proteins are those involved in the carcinogenic phenotype. Diet has also been shown to affect proteins involved in inflammation. A soy intervention in women increased proteins with anti-inflammatory functions. A high-fat diet increased COX-2 protein by 45% in the rat colon. Even body weight can affect COX-2, and COX-2 mRNA in healthy colorectal mucosa was almost 2.6-fold higher in overweight and obese persons than in normal-weight persons. Importantly, changes in proteins may be more valuable than changes in eicosanoids, which can be transiently formed in cells, indicating a need for future investigations of proteins affected by a Mediterranean diet. Proteomic studies are already under way in investigations of colon carcinogenesis. Targeted proteomics of the proteins expected to be affected by a Mediterranean diet should now be undertaken to develop a better understanding of the mechanisms that tie this diet to colon cancer risk.

CONCLUSION

In summary, the levels of proteins involved in uptake, trafficking, and metabolism of fatty acids can be expected to be altered by the Mediterranean diet. These proteins can contribute to interindividual variability in the levels of fatty acids and eicosanoids. A proteomic analysis of colonic mucosa would allow for a pathway-based approach of fatty acid metabolism. The relative levels of proteins may not be strictly predictive of enzymatic activity, but determining such levels would be an important step towards understanding how a Mediterranean diet can influence the risk of colon cancer. The identified proteins may then be good candidates for further study with regard to regulatory factors such as genetic polymorphisms and their subsequent impact on carcinogenesis. These kinds of approaches all have great potential for increasing understanding of how interindividual variability in colonic fatty acids affects inflammation and the risk of colon cancer.

Acknowledgments

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Figure 1. Metabolism of dietary fatty acids to eicosanoids
Metabolic processes modulate the effect of dietary changes on fatty acid levels in membranes and on the eicosanoids that are formed.
**Figure 2. Formation of eicosanoids: select products**

The other products shown are from n-6 fatty acids except for PGE3 and LTB5, which are formed from n-3 fatty acids. Eicosapentaenoic acid inhibits oxygenation of arachidonic acid by cyclooxygenase 1 (COX-1), but this inhibition is modest with COX-2. The n-9 fatty acid oleic acid (18:1, n-9) forms 20:3 (n-9), which has not been studied as extensively (not shown), but it has been reported to form the unstable LTA3 via 5-lipoxygenase that, in turn, inhibits formation of LTB4.
Table 1

Dietary intakes and changes in levels of fatty acids in blood in select Mediterranean dietary interventions that successfully increased monounsaturated fatty acid (MUFA) intakes and reported levels of fatty acids in blood. The “Change” columns show either the change from baseline or differences versus a control group, depending on how it was reported in each publication. The dietary fat intakes used for the calculation were expressed as percentage of energy intake.

<table>
<thead>
<tr>
<th>Study</th>
<th>Intervention</th>
<th>Change in diet</th>
<th>Change in blood fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>de Lorgeril et al. (1998)* Lyon Diet Heart Study</td>
<td>Provided counseling and a high-MUFA spread</td>
<td>SFA: 37%↓</td>
<td>SFA: 2%↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18:1, n-9: 16%↑</td>
<td>18:1, n-9: 12%↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PUFA, n-6: 23%↓</td>
<td>PUFA, n-6: 8%↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>PUFA, n-3: 12%↑</td>
</tr>
<tr>
<td>Djuric et al. 173,174 (2009)† Mediterranean Eating Study</td>
<td>Provided exchange-list counseling</td>
<td>SFA: 21%↓</td>
<td>SFA: 5%↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MUFA: 59%↑</td>
<td>MUFA: 25%↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PUFA: 21%↓</td>
<td>PUFA: 1%↑</td>
</tr>
<tr>
<td>Paniagua et al. (2007)‡,167</td>
<td>Provided all food</td>
<td>SFA: 61%↓</td>
<td>SFA: 8%↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MUFA: 156%↑</td>
<td>18:1, n-9: 22%↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PUFA: 0%↓</td>
<td>18:2, n-6: 4%↓</td>
</tr>
<tr>
<td>Urquiaga et al. (2004)§,200</td>
<td>Provided all food</td>
<td>SFA: 37%↓</td>
<td>SFA: 4%↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MUFA: 7%↑</td>
<td>MUFA: 11%↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PUFA: 2%↓</td>
<td>PUFA: 7%↑</td>
</tr>
<tr>
<td>Vessby et al. (2001)¶ KANWU study168</td>
<td>Provided fats as well as counseling about high-MUFA diet</td>
<td>SFA: 28%↓</td>
<td>SFA: 2%↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MUFA: 62%↑</td>
<td>18:1, n-9: 10%↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PUFA: 2%↓</td>
<td>18:2, n-6: 9%↓</td>
</tr>
<tr>
<td>Vincent-Baudry et al. (2005)*** Medi- RIVAGE Study175</td>
<td>Provided counseling</td>
<td>SFA: 31%↓</td>
<td>16:0: 6%↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MUFA: 9%↑</td>
<td>18:1, n-9: 3%↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PUFA: 7%↑</td>
<td>18:2, n-6: 1%↓</td>
</tr>
<tr>
<td>Zazpe et al. (2008)†† PREDIMED Study201</td>
<td>Provided counseling</td>
<td>SFA: 5%↓</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MUFA: 11%↑</td>
<td>18:1, n-9: 2%↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PUFA: 3%↑</td>
<td>–</td>
</tr>
</tbody>
</table>

* For the study of de Lorgeril et al., data from individuals without cancer was used, and the difference between the intervention group and the control group is shown. Dietary intakes of n-3 PUFA were not given. Fatty acids were measured in plasma 2 months after randomization.

† Data are for the decrease over 6 months in the intervention arm (percent change from baseline). Fatty acid levels were those measured in total fasting plasma phospholipids.

‡ Differences between a high-MUFA diet and an SFA diet are shown after 28 days. Fatty acid levels were those measured in plasma phospholipids.

§ Differences are shown between a Mediterranean diet (27% of energy from fat) and an Occidental diet (40% of energy from fat) after 30 days of feeding. Fatty acids were measured in total plasma.

¶ Change is from baseline to mean over 90 days of treatment. Fatty acid analysis was of serum phospholipids.

*** Data are for change from baseline to 3 months in the Mediterranean intervention group. Fatty acids were those measured in fasting plasma.

†† Data are for change from baseline to 12 months in the Mediterranean plus olive oil group. Fatty acids were those measured in fasting plasma.

Abbreviations: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.
Table 2
Phospholipid fatty acids measured in normal human colonic mucosa from eight healthy subjects.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Mean* (% of fatty acids)</th>
<th>Standard deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>20:4, n-6</td>
<td>6.8</td>
<td>1.2</td>
<td>3.5–8.8</td>
</tr>
<tr>
<td>20:5, n-3</td>
<td>2.3</td>
<td>0.8</td>
<td>0.5–3.6</td>
</tr>
<tr>
<td>18:2, n-6</td>
<td>16.1</td>
<td>2.2</td>
<td>9.2–18.4</td>
</tr>
<tr>
<td>18:1, n-9</td>
<td>26.8</td>
<td>2.2</td>
<td>23.8–31.7</td>
</tr>
<tr>
<td>18:0</td>
<td>14.8</td>
<td>2.8</td>
<td>8.3–22.2</td>
</tr>
<tr>
<td>16:0</td>
<td>24.7</td>
<td>4.6</td>
<td>19–32.3</td>
</tr>
</tbody>
</table>

* Adapted from Nishida et al. 182 with permission from BMJ Publishing Group Ltd.