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Omega-3 PUFAs and gut microbiota: A preventive approach for colorectal cancer

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Abstract

The influence of diet on the composition of the intestinal microbiota and related pathologies has been known for some time. Some classes of nutrients, such as fatty acids belonging to the omega 3 series, have particular effects on the bacteria that make up the intestinal microbiota. ω-3 PUFAs affect the gut microbiota in three different ways: by modulating the type and abundance of intestinal bacteria, by regulate SCFAs levels, and by alter the levels of proinflammatory mediators. Through these modalities, ω-3 PUFAs could be useful for the prevention of intestinal diseases such as Colorectal Cancer (CRC). The ability of ω-3 PUFAs to modulate the intestinal inflammatory response, to preserve the integrity of the intestinal mucosa and to modulate the bacterial composition of the intestine, could be useful as a preventive strategic approach to hinder the development of CRC.

Introduction

The gut microbiota consists of a population of various and highly dynamic microorganisms, whose composition is characterized by the presence of 500 to 1,000 different bacterial species, and a low number virus and fungal species.[1,2] Our intestine hosts trillions of microorganisms, the highest concentration ($9x10^{13-14}$) being found in the colon, with a very high bacterial diversity.[1,3] The main bacteria present in the colon belong to four main phyla: Firmicutes (64%), Bacteroidetes (23%), Actinobacteria and Proteobacteria.[4] The intestinal microbiota is formed at the time of birth, during the passage through the birth canal. At this time, the neonatal intestine is colonized by the maternal flora, composed of *Bacteroidetes*, *Bifidobacterium*, *Prevotella* and *Lactobacillus* spp.[3,5] The intestinal microbiota regulates several important biological processes, including fermentation of amino acids and saccharides with the subsequent production of Short-Chain Fatty Acids (SCFA), succinate, ethanol, amines, lactate, phenols, thiols and indoles, of hydrogen gas (such as H2S acetate and methane)**,** degradation of undigested proteins and carbohydrates, and the transformation of bile acids.[3,5] Furthermore, gut microbiota plays a fundamental role for the health of our organism. It regulates the proliferation of pathogenic bacteria present in the intestinal tract (such as *Clostridia* or *Colibacillacea*), stimulates the immune system, regulates the absorption of nutrients, regulates host metabolism, promote the production of vitamins such as vitamin K and biotin, and enzymes and the synthesis of compounds useful for the trophism of the colon mucosa and that are essential for cell renewal.[3,5,6] On the other hand, any alteration in intestinal eubiosis can lead to an altered composition of gut microbiota, known as dysbiosis, a condition that determines changes of the tight intercellular junctions responsible for maintaining the integrity of the intestinal mucosa and its permeability which is fundamental to prevent the access of pathogens.[6] In case of severe dysbiosis, Mucosal Associated Lymphatic Tissue (MALT) once activated triggers the inflammatory cascade (leukocytes, cytokines, TNF-α) and consequently a massive tissue injury and a possible fostering of colorectal carcinogenesis process.[7] Dysbiosis develops due to a variety of factors including physical and psychological stress, environmental stress, diet, and drug intake.[3] In particular, diet appears to be one of the determining factors for the composition of the intestinal microbiota.[3,8] A diet rich in saturated fats and simple sugars and a poor content of fruits and vegetables is known to increase the risk of metabolic disorders.[8] Furthermore, it is well established that

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diet has a great influence (57% vs 12% for genetic factors) on the composition of the microbiota. Many studies have shown that long-term consumption of large amounts of fat and sugar results in a worsening of dysbiosis and in a changed composition of the microbiota, as well as in the production of endotoxins.[9] Moreover, this type eating-habit leads to changes in the intestinal mucosa, which becomes thinner and thinner, more permeable to pathogens and antigens and more susceptible to the establishment of low-grade but persistent inflammation.[3,9]

The association between diet-induced dysbiosis and Colorectal Cancer (CRC) was highlighted by a study of the intestinal microbial structures and faecal metabolite profiles among populations consuming different types of diet.[10] For instance, the intestinal microbiota of rural Africans, whose diet is rich in fibre and low in fat, is characterized by a predominance of the genus *Prevotella*, involved in the degradation of starches, hemicelluloses and xylans.[10] On the other hand, the microbiota of the American population is predominated by the *Bacteroides* genus with an increased abundance of potentially pathogenic proteobacteria, such as *Escherichia Coli* and *Acinetobacter*.[10] These microbial and structural differences parallel the low incidence of colorectal cancer in Africa compared to Western countries.[10] There are also differences in faecal metabolites profile, with higher SCF in Native Africans and higher secondary bile acids in African Americans.[10] These observational results were consistent with those from a study showing that the switching of African-Americans diet to a high-fibre, low-fat diet for 2 weeks, increased SCFA production, suppresses secondary bile acid synthesis, and reduces inflammation of the colon mucous membranes and proliferation of tumour markers. In contrast to the tumour, promoting effects of secondary bile acids, SCFs have been shown to protect against colorectal cancer through their beneficial effects on immune and metabolic pathways.[10]

Other studies have suggested that a compromission of the colonic mucus layer, a physical barrier that separates trillions of gut bacteria from the host, may represent a potential mechanism by which a direct interaction between Western diet and gut microbiota can foster carcinogenesis.[11,12] In fact, the excess of saturated fats and refined sugars slows the growth rate of mucus and increases the penetration of the intestinal barrier by bacteria.[11,12] It also causes a gradual decrease in SCFA-producing bacteria, such as *Bifidobacterium* and *Bacteroidales*, and an increase in *Firmicutes*.[11,12]

Given the important role of the diet on intestinal dysbiosis and the genesis of CRC, recent studies have investigated the role of particular polyunsaturated fatty acids present in the diet on the intestinal microbiota and on colorectal carcinogenesis.

In this review we will talk about one of the fundamental components of the Mediterranean diet, the fatty acids of the omega 3 series, their role in carcinogenesis and their interaction with the intestinal microbiota.

ω-3 PUFA: characteristics and role in pathological conditions

Long-chain Polyunsaturated Fatty Acids (PUFAs) are organic substances characterized by a high number of carbon atoms, some of which are linked with a double bond (hence the definition of unsaturated fatty acids).[13] From a nutritional point of view, the most important PUFAs are represented by omega 3 (ω-3) series, which include α-linolenic (ALA; 18:3), eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6). Fishery products from fatty fish such as salmon, sardines, mackerel and blue fish in general, are, by far, the most important source of ω-3 PUFA, as absolute quantity and in percentage.[13] EPA and DHA come mainly from the consumption of fish that, thanks to a diet based on microalgae rich in DHA and EPA and phytoplankton, accumulates ω-3 PUFA.[1] ALA is mainly obtained through the intake of flax seeds, chia seeds and walnuts. EPA and DHA can be synthesized in the human body by using ALA as a precursor. However, this conversion account only for 6% of EPA and <4% of DHA.[1] For this reason, ω-3 PUFAs are considered essential fatty acids to be taken through the diet.[1] The interest in the ω-3 PUFA fatty acids originated from the epidemiological observations of the populations of the west coast of Greenland,[14,15] whose diet is characterized by a high fat content and a reduced consumption of fruit and vegetables. Despite this type of diet, which is markedly different from the Mediterranean diet, these populations have a reduced incidence of dyslipidemia and mortality from myocardial infarction compared to other European populations.[13] The explanation for this phenomenon lies in the fact that the high consumption of fish, and therefore ω-3 PUFA, results in a higher content of EPA and a lower content of Arachidonic Acid (AA) in the membrane phospholipids of platelets.[13] Since TXA3, a non-aggregatory thromboxan, is formed from EPA, the increase in the EPA/AA platelet ratio leads to a lower efficiency of platelet aggregation, a lower tendency to form thrombi and, consequently, a lower incidence of atherosclerosis.[13] Therefore, the administration of ω-3 PUFA may reduce the progression of atherosclerotic lesions and improve the haemodynamic and physical properties of the large arteries.[13] According to the Italian Society of Human Nutrition (SINU), the levels of reference intake for the Italian population (LARN) for ω-3 PUFAs[16] are those shown in Table 1.

Studies carried out on the populations of Greenland,[14,15] have shown, in addition to the usefulness of ω-3 PUFAs in reducing the risk of coronary heart disease,[17] these molecules are also effective in improving parameters associated with the Metabolic Syndrome (MetS).[18] MetS is a condition characterized by the combination of three or more different components of cardiometabolic risk factors, including obesity, hypertension, dyslipidemia and impaired glucose tolerance.[18] Patients with MetS are at high risk of cardiovascular disease and type 2 diabetes (DM-2).[19] As the mechanisms responsible for MetS involves both genetic and acquired factors, an effective therapeutic strategy in MetS patients may be based on lifestyle modifications, weight loss and a regular physical activity associated with the administration of supplements, in particular PUFA ω-3.[19,20] Consistent with these observations, data from some Randomized Control Trial (RCT) have shown that a 12 weeks supplementation with ω-3 PUFA (1.2 g/day) resulted in a significant reduction of circulating concentrations of triglycerides and non-esterified fatty acids while, the prevalence of MetS decreased by 10.5%.[21,22] Another RCT in which patients with MetS were given 3 g of fish oil up to 90 days, reported, a significant reduction in diastolic blood pressure compared to the baseline value in these patients.[19] Other studies have also provided evidence on the use-

Table 1. LARN for ω-3 PUFAs.

Age	mg/day
Infants	EPA-DHA 250 mg+ DHA 100 mg
Children - teenagers	EPA-DHA 250 mg1-2 anni +DHA 100 mg
Adults - elderly	EPA-DHA 250 mg
Pregnancy - breastfeeding	EPA-DHA 250 mg+DHA 100-200 mg

fulness of ω-3 PUFAs in the treatment of mood disorders, as their intake is correlated with a decrease in anxiety symptoms.[23] These findings have suggested the clinical use ω-3 PUFAs also in the treatment of major depressive disorder.[24]

Interestingly, recent experimental studies have suggested a possible anticancer activity of ω-3 PUFAs.[25] In particular, these studies have provided evidence on the capability of ω-3 PUFAs to inhibit some of the multiple oncogenic pathways which lead to the inhibition of proliferation and activation of apoptosis.[25,26] In this setting, recent in vitro studies have highlighted the fact that, ω-3 PUFAs may thwart the growth of hormone resistant tumours.[1,26] Furthermore, these studies also showed a preferential inhibition of ω-3 PUFAs for triple negative breast cancer cells rather than luminal breast cancer cells.[1,27] Modulation of molecular pathways promoting inflammation is another supposed mechanism by which ω-3 PUFAs can exert anticancer effects.[28] Consistent with these observations, some, in vivo studies have reported that Specialized Pro-resolving lipid Mediators (SPMs), which include EPA and DHA-derived resolvins, protectins and maresins, may inhibit chemically-induced colitis in rodents. These SPMs act by suppressing NF-kB nuclear translocation, thus reducing the expression of TNF-α and other pro-inflammatory cytokines while, they increase the production of anti-inflammatory cytokines, such as interleukin-10, in monocytes.[29-32]

SPMs also regulate intestinal bacterial populations by limiting collateral tissue damage that often occurs during the elimination of bacterial pathogens. They also stimulate the production of antimicrobial peptides in the intestinal mucosa, can improve phagocytosis of bacteria by inhibiting the production of interleukin- 12, and suppress the migration of dendritic cells.[29,30,33]

ω-3 PUFA: influence on the intestinal microbiota

Like all dietary components, ω-3 PUFAs may also influence the composition of the intestinal microbiota. It has previously been observed that -3 PUFAs play a role in the regulation of intestinal bacterial populations and in the promotion of intestinal immune homeostasis, through their endogenous metabolites.[33]

ω-3 PUFAs affect the gut microbiota in three different ways; first, by modulating the type and abundance of intestinal bacteria. For example, compared to saturated fatty acids, ω-3 PUFAs influence positively the intestinal microbiota by increasing the presence of Lactobacillus and Bifidobacteria.[34-37] This phenomenon has been reported to be associated with a low-grade inflammation, and a lower presence of Helicobacter and Fusobacteria nucleatum.[34-37]

Furthermore, ω-3 PUFAs could exert beneficial effects on the intestinal microbiota by decreasing the growth of *Enterobacteria*, by increasing the growth rate of *Bifidobacteria*, and subsequently, by inhibiting the inflammatory response associated with metabolic endotoxemia.[38] ω-3 PUFAs derived from the diet, are partially metabolized in the distal intestine, by anaerobic bacteria such as *Bifidobacteria* and *Lactobacilli*.[39] Experimental in vivo studies in animal models have shown that these metabolic processes determine marked changes in the microbial composition of the intestine.[39] The increase in the population of beneficial bacteria results in an improvement of the intestinal microenvironment and the barrier function of the intestinal mucosa, an increase of the thickness of the intestinal mucosa, and the fostering of weight loss by modulating the expression of genes related to fat metabolism.[40] The effects of ω-3 PUFAs on the intestinal microbial population also result in the prevention and/or improvement of specific symptoms of various diseases related to intestinal dysbiosis. *Firmicutes* and *Bacteroidetes* are two major dominant bacterial *phyla* of the human gut microbiota.[41]

The *Firmicutes*-to-*Bacteroidetes* ratio (F/B ratio) has been reported to be associated with obesity.[42] In fact, while in healthy subjects this ratio ranges from 1:1 to 3:1, in 35% of obese or overweight subjects there is an unbalanced ratio to favour of the *Firmicuti*, from 3: 1 up to 25: 1.[43] On the other hand, Onishi *et al.*[44] have shown that ω-3 PUFAs are able to hinder the decrease in the F/B ratio observed in mice fed a high-fat diet. A second way in which ω-3 PUFAs may affect the intestinal microbiota is related to their capability to alter the levels of proinflammatory mediators, such as endotoxins, resulting from bacterial Lipopolysaccharide (LPS) and Interleukin-17 (IL-17).[45,46] Following the occurrence of dysbiosis, LPS can cross the intestinal wall, due to the loss of the integrity of the intestinal barrier, thus causing further damages. The increased intestinal permeability, in turn, results in an accumulation of toxic bacterial products such as LPS and bacterial DNA in the systemic circulation. This phenomenon can potentially elicit an inflammatory response.[45,46] Consumption of ω-3 PUFAs inhibits LPS-induced pro-inflammatory cytokine production in human blood monocytes, relieving intestinal inflammation, by inhibiting LPS induced activation of NF-κB signalling pathways.[47] These observations are in line with the findings from other in vitro studies showing that macrophage incubation with ω-3 PUFAs reduces LPS-induced MAPK activity and decreases the expression of proinflammatory mediators, such as TNF-α.[48] ω-3 PUFAs also promote the release of large amounts of anti-inflammatory cytokines from resident macrophages, such as Interleukin-10 (IL-10), the induction of regulatory T lymphocytes (Treg) and prevent an excessive development of T-helper 17 cells (Th17) and that of IL-17, a pro-inflammatory cytokine produced mainly by Th17 cells, which causes tissue inflammation. Therefore, ω-3 PUFAs can reduce intestinal inflammation by increasing the differentiation of Tregs and by decreasing the production of IL-17.[49] A third way in which ω-3 PUFAs may affects gut microbiota relies on the ability of these molecules to regulate SCFAs levels.[37] The production of SCFAs, including butyrate, propionate and acetate, plays an essential role in maintaining a healthy mucosa and in the production of anti-inflammatory interleukins. SCFAs derives from the bacterial fermentation of carbohydrates from food. This process occurs in the colon, under anaerobic conditions, with the contribute of *Lactobacilli* and *Bifidobacteria*. Among the SCFAs, butyric acid is the preferred energy source for colon cells, where it controls the state of inflammation, proliferation, differentiation and apoptosis. It also has a role in strengthening the defensive barrier of the colon by increasing the production of mucin and antimicrobial peptides and, by decreasing the permeability of the intestinal epithelial and by increasing the expression of the proteins constituting the tight junctions.[3] Numerous studies have shown that increasing the daily intake of 4 g of mixed ω-3 PUFAs (DHA and EPA) significantly increased the density of butyrate-producing bacteria.[37] The mechanisms mentioned above could be useful in the prevention and adjuvant treatment of CRC.

ω-3 PUFAs: role in the prevention of colorectal cancer

The ability of ω-3 PUFAs to modulate the intestinal inflammatory response, to preserve the integrity of the intestinal mucosa and to modulate the bacterial composition of the intestine, could be

useful as a preventive strategic approach to hinder the development of CRC. This malignant disease represents one of the most common cancers in the world population, with a multifactorial pathogenesis linked, among other things, to environmental factors, a sedentary lifestyle and an incorrect diet.[50] The inverse correlation between ω-3 PUFA intake and CRC development has been highlighted by several human studies that have analyzed the relationship between microbiota and ω-3 PUFA in some stages of carcinogenesis.[51] In this setting, Prossomariti *et al.*,[52] showed that in patients with ulcerative colitis (RCU, a disease which is serious risk factor for CRC), supplementation with EPA (2 g/day for 90 days) led to an improvement in endoscopic inflammation and to the modulation of the intestinal microbiota. In particular, the genus *Parabacteroides*, which has been found to be lower in patients with RCU, was significantly increased after EPA supplementation. Conversely, *Clostridium* spp. and *Bacteroides*, which are known to trigger mucolytic metabolism, were found to be decreased after supplementing with EPA.[52] Other numerous epidemiological and preclinical studies further support the preventive effects of ω-3 PUFA on CRC. For example, in the VITamins And Lifestyle (VITAL) cohort study, subjects who routinely took fish oil supplements had lower risks of developing CRC than those who didn't take supplements.[53,54] Another study, carried out by the European Prospective Investigation into Cancer and Nutrition (EPIC) showed that increased consumption of ω-3 PUFA reduces the risks of CRC.[55] The results of a randomized, double-blind, placebo-controlled study, demonstrated that EPA intake was associated with a reduced number and size of polyps in patients with Familial Adenomatous Polyposis (FAP).[56] In particular, this study showed that adding EPA at a dose of 2 g/day for 6 months reduced the number and size of polyps by 20-30%. These results, are comparable to that obtained by other studies following long-term treatments with cyclooxygenase-2 inhibitors.[11] Besides CRC prevention, ω-3 PUFAs have been reported to be useful as adjuvants to chemotherapy in the treatment of advanced disease.[57] In this context, clinical observations have reported that the increased ω-3 PUFA intake was also associated with improved disease-free survival in patients with stage III CRC.[57]

Moreover, the results from a double-blind, randomized, placebo-controlled phase II study, carried out by Cockbain *et al.*[58] demonstrated that the oral administration of EPA to patients with advanced CRC who underwent liver resection due to liver metastases was associated with increased overall survival. Furthermore, some clinical investigations have also shown a significant correlation between ω-3 PUFA intake and survival of CRC patients.[34] Finally, a study carried out on two groups of women that were treated with <0.1 g/day or with 0.3 g/day of ω-3 PUFA respectively, showed a decreased rate of mortality in subjects administered with the highest dose. This reduction was more marked when ω-3 PUFA consumption was further increased to 0.15 g/d.[34]

In recent years we have also seen how nutrition, including the administration of ω-3 PUFA, is able to influence the health conditions of individuals and the susceptibility to diseases by defining the metabolic response and gene expression. In fact, epigenetic modifications can play a significant role in the onset and pathogenesis of CRC, with mechanisms such as DNA methylation and chromatin remodeling. ω-3 PUFA, however, act differently as their epigenetic action does not involve DNA methylation. In fact, it has been seen that the intake of PUFA in association with dietary fibres upregulates the nuclear receptors dependent on the EPA and DHA ligand and supports the increase in butyrate production through the fermentation of fibres, which directly and indirectly modifies the acetylation of histones. These combinatorial effects improve the

mitochondrial shuttle of L-carnitine, inhibit lipogenesis and promote the accumulation of acetyl CoA-dependent beta-oxidation. The increased beta-oxidation of the mucosa maintains a hypoxic environment in the intestine and promotes the growth of obligate anaerobic bacteria and inhibits dysbiotic microbial expansion.[59] This paves the way for future studies on ω-3 PUFA, CRC and epigenetic modifications.

Conclusions

Daily consumption and the possible integration of ω-3 PUFA appears to exerts beneficial effects on human. Primary and secondary prevention against cardiovascular and ischemic diseases can be made by maintaining adequate circulating levels of ω-3. This adaptation is also effective to improve the parameters related to cholesterol and triglycerides. Adequate consumption of ω-3 PUFA may also improves the blood and pressure parameters of subjects affected by MetS, while it is still not clear whether the additional supplementation with ω-3 PUFA alone can have preventive effects on the development of MetS. The studies that correlate the consumption of ω-3 PUFA to the inhibition of carcinogenesis, and in particular to CRC, are interesting. This multifactorial pathology recognizes, among its causes, an alteration of the intestinal microbiota, referred as dysbiosis. ω-3 PUFAs influence the intestinal microbiota through three different mechanisms: i) by modulating the type and quantity of bacteria present in the intestine; ii) by reducing the inflammatory phenomena caused by intestinal dysbiosis; iii) by regulating the levels of SCFAs in the intestine. These effects have been also shown to be effective in the prevention and/or treatment and survival of CRC. In fact, it has been observed that the incidence of CRC is lower in patients who habitually consume ω-3 PUFA. However, these results do not prove a direct relationship between ω-3 PUFA and prevention of CRC as several other factors may contribute to the etiopathogenesis of CRC. It was also possible to observe how patients with CRC or FAP had beneficial effects in terms of reduction of neoplastic and paraneoplastic lesions and in terms of survival. We can therefore conclude that the dietary intake and the possible daily supplementation of ω-3 PUFA have beneficial effects on human health.

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