The Effect of L-Carnitine on Colorectal Cancer: A Review on Current Evidences

Fatemeh Radkhouy[1], Samira Soltanieh[1], Shakiba Solgi[1], Maedeh Ansari[1], Behnood Abbasi [1]*

[1] Department of Nutrition, Science and Research Branch, Islamic Azad University, Tehran, Iran

Abstract

Colorectal cancer is the most common type of gastrointestinal cancer that results from abnormalities or changes in the genome and uncontrolled cell proliferation. Carnitine is a potent antioxidant that may result in an increase in cellular respiration, apoptosis, a reduction in proliferation and inflammation of tumor cells by various mechanisms. The present study was conducted to summarize the effects of carnitine on the treatment or prevention of colorectal cancer. Electronic literature searches were conducted on Medline, Web of Science and Google Scholar until May 2018. Our search was supplemented with the search of publisher databases Elsevier, Wiley Online and SpringerLink and for any pertinent studies, we screened the references of all included studies. There were no restrictions regarding the language of publications. The search was conducted with the following words "L-Carnitine", in combination with Colorectal Cancer, Neoplasm, Colon, Rectum, Apoptosis, Inflammation and "Precancerous Lesions" among animal and *in vitro* studies. From six interventional studies investigated in this article, one of them was performed on two groups of mice having precancerous lesions and macroscopic colonic tumors divided into AOM and APC groups and five other studies on adenocarcinoma cell lines of NCOL-1, CACO-2, HT-29, and SW480. One of them also was performed on DMH-induced colon carcinogenesis mouse model. These studies reported significant increment in the amount of the fatty acid transportation into the mitochondria; generation of mitochondrial superoxide anions (O^{2-}), apoptosis and cell death in cells which were exposed by carnitine. An increment was also observed in pro-apoptotic proteins Caspase, Bak and Bax and reduction in anti-apoptotic proteins Bcl-Xl. In these studies, cellular inflammation which was associated with products of the cyclooxygenase enzyme pathway, and cancer cell proliferation was reduced as well and there was an increment in DNA fragmentation. The aberrant crypt foci development and pre-cancerous lesions were significantly inhibited by carnitine in the colons of the studied mice but did not exert protective effects on their intestinal tumors. Carnitine may have potential anticancer effects and inhibits the progression of macroscopic and pre-cancerous tumors and prevents the growth and proliferation of cancerous cells.

Keywords: *Colorectal Cancer, Carnitine, Butyrate, Apoptosis, Neoplasm*

Review Article

Received Date: Jun.1.2018
Revised Date: Jul.15.2018
Accepted Date: Nov.1.2018

✉**Correspondent Author:**
Dr. Behnood Abbasi Department of Nutrition, Science and Research Branch, Islamic Azad University, Tehran, Iran.
abbasi.b@srbiau.ac.ir

Introduction:

Today cancer is one of the greatest problems in global health[1]. 8.8 million people worldwide died from cancer in 2015 and nearly one out of every six deaths are caused by cancer[2]. After cardiovascular disease and injuries, cancer is the third leading cause of death in Iran with the prevalence of 13 % [3]. Colorectal cancer is one of the most important cancers in all over the world [4]. This disease is considered the most common gastrointestinal cancer in the world [5]. This disease has got an annual incidence of more than 1.2 million people in the world[6].

Colorectal cancer is the most common Gastrointestinal tract malignancy in western countries and is the second leading cause of cancer mortality[7]. According to WHO reports in 2014, colorectal cancer with an annual prevalence of 3300, after breast cancer, is the second most common cancer among women in Iran. Also, it is the fourth most common cancer among men after gastric, bladder and prostate cancer, with an annual prevalence of 3800 [8]. This disease accounts for 8.5% of cancer deaths in women and 7.5% of deaths from cancer in men [8]. Treatment of cancer and its costs is one of the main problems of the health system all over the world including in Iran [9]. According to WHO statistics in 2010, the total annual economic cost of cancer was estimated to be approximately 1.16 trillion US$ [10]. The higher rate of incidence of cancer in young people, high prevalence of colorectal cancer in under 50-years population in Iran and the postponed referring and diagnosis of the disease; may seriously affect health system of Iran [11]. Also, the reduction in gross national product (GNP) rate due to the loss of productivity, disability and the early death of cancer; imposes great economic costs on individuals and society [12].

Colorectal cancer is a genetic disorder that results from abnormalities or changes in the genome which ultimately may result in uncontrolled cell proliferation or carcinoma [13]. Ages older than 50 years, family history of colorectal cancer, increased carbohydrate intake, consuming higher calories from animal sources, higher intake of unsaturated animal fats and unsaturated vegetable oils, and also lower consumption of dietary fiber as a result of low vegetable intake, consumption of alcohol, smoking, low physical activities, and obesity; are the risk factors for colorectal cancer[4,14-16]. Higher metabolism of arachidonic acid in the cyclooxygenase pathway also leads to the production of different kind of prostaglandins and thromboxanes, which may result in the initiation or progression of colorectal cancer [17]. L-carnitine is a nonessential amino acid derived from methionine and lysine amino acids, that its limited amounts can be produced endogenously in the liver, kidneys and the brain[18,19]. It also can be obtained from dietary sources, especially meat and dairy products[20]. L-carnitine is absorbed from the intestine and transmitted to other tissues by specific carriers[18]. L-carnitine and its short-chain derivatives are essential cofactors in the metabolism of fats and are necessary factors in cellular energy production. Carnitine transmits long-chain fatty acids across the internal membrane of mitochondria. These fatty acids enter the beta-oxidation reactions after being transferred[21,22]. Carnitine is a potent antioxidant as well and absorbs active oxygen species in the mammalian tissues, therefore it might have anticancer properties[21,23-25]. Carnitine may also be able to increase cellular respiration and apoptosis, decrease the proliferation of cancer cells and reduce inflammation by various mechanisms[19,25-27]. In this regard, Elmirini *et al.* showed that acetyl-carnitine and butyrate potentially have anti-cancer properties that inhibit the growth of colon cancer cells in vitro [28]. Roscilli *et al.* Also referred to the anti-tumor effects of carnitine in their research [29]. Dionne *et al.* in their experiments observed that carnitine may be able to inhibit the development of pre-cancerous lesions and macroscopic colonic tumors as well [19].

According to the growing incidence of colorectal cancer, this disease is a major problem in cancer management in Iran. Since there are considerable costs for the treatment of this disease and it also has high prevalence and due to its effects on reduction of economic productivity; Therefore, the present review article was conducted to assess the effectiveness of carnitine supplementation on the prevention of the development of pre-

cancerous lesions or treatment of colorectal cancer in previous clinical trials.

Materials and Methods:

Electronic literature searches were conducted on Medline, Web of Science and Google Scholar until May 2018. Our search was supplemented with the search of publisher databases Elsevier, Wiley Online and SpringerLink and for any pertinent studies, we screened the references of all included studies. There were no restrictions regarding the language of publications. The search was conducted with the following words "L-Carnitine", in combination with Colorectal Cancer, Neoplasm, Colon, Rectum, Apoptosis, Inflammation and "Precancerous Lesions" among Animal and in vitro studies. Eligibility criteria included: experimental studies published in peer-reviewed journals and studies that used carnitine supplementation in any dose. Duplicates were removed (2 articles); the relevant papers were selected in three phases. In the first and second phases, titles and abstracts of papers were screened and irrelevant papers were excluded. In the last phase, the full text of recruited papers was explored intensely to select only relevant papers. After excluding the studies that did not report our primary outcome or fit the criteria, six suitable studies were identified for review. Our primary outcomes were enzymatic and pathologic markers of apoptosis.

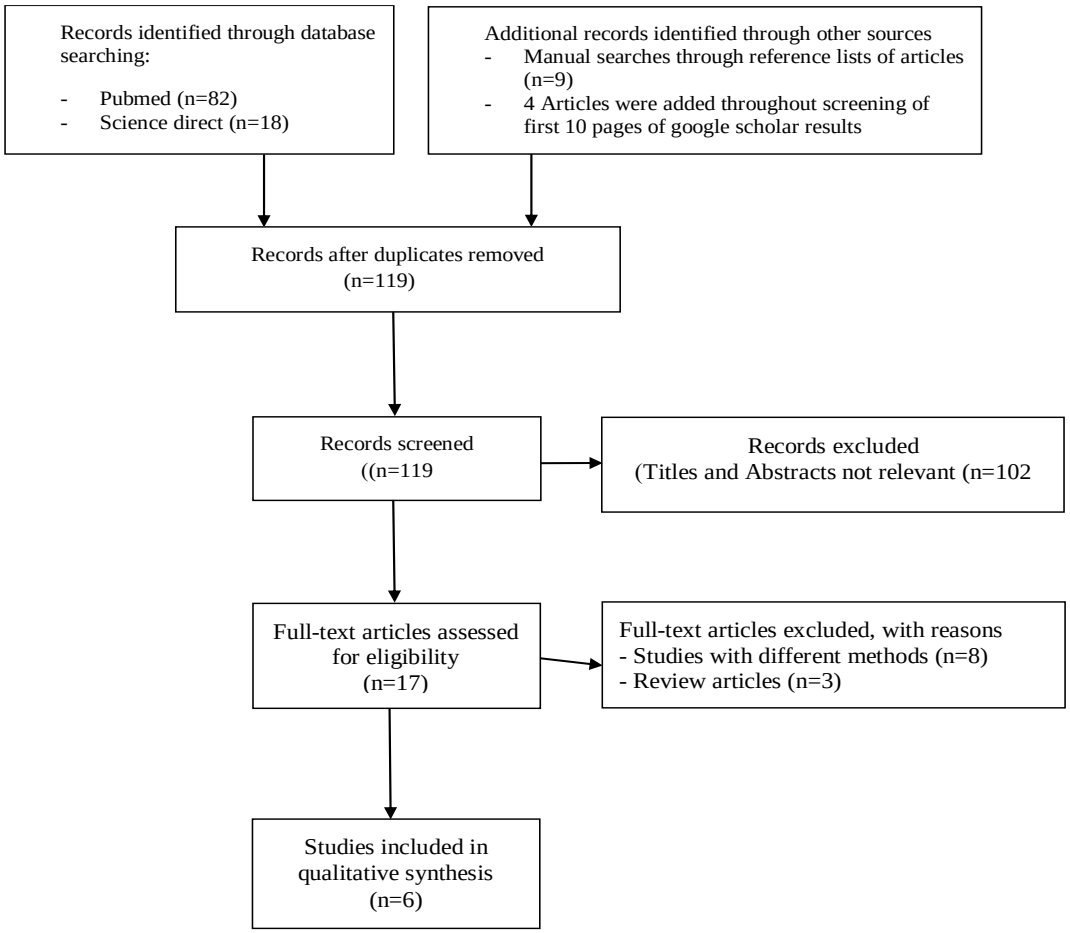

Figure 1: Identification of publications

Results:

In this study, six articles were investigated (Figure 1). These articles were conducted during 2005 to 2015 and examined the role of carnitine supplementation in the development of pre-cancerous lesions, the progression of macroscopic tumors and the effect of carnitine

on changes in the concentration of factors affecting apoptosis and cell death, such as Caspase-3, Bcl- xL, Bax & Bak in cancer cells. The effects of carnitine on cellular inflammation related to cyclooxygenase enzyme pathway products and the effects of carnitine on reducing the proliferation of cancer cells and the factors affecting the destruction of DNA of these cells has also been studied in these articles. These articles have also examined the amount of fatty acids transported into the mitochondria and the amount of superoxide anion (O2) produced in the mitochondria, which are among the important factors in the apoptosis of cancer cells[19,25-27].

In a study by Dionne *et al.* two groups of mice were divided into AOM and APC groups, each of which had pre-cancerous masses. They were provided with high-fat diets with or without carnitine. This study was performed to evaluate the effect of carnitine on the progression of these lesions[19]. Five other studies have been conducted on different adenocarcinoma cell cultures of HT-29, NCOL-1, CACO-2 and SW 480 which were incubated with carnitine or in combination with some other chemicals such as butyrate and curcumin, to demonstrate the effects of carnitine on the progression of cancerous tumors in this cell cultures[25-29]. Roscili et.al also performed a part of their study experimentally on mouse groups [29] (Table 2).

Aberrant Crypt Foci, Crypt Multiplicity, and Macroscopic Tumors

The results of a study by Dionne *et al.* done on development of precancerous lesions and progression toward macroscopic tumors showed that the addition of carnitine to high-fat diets of mice significantly inhibited ACF (4.9±0.7 vs 9.3±0.88, P<0.001) , crypt multiplicity 1.6±0.08 vs. 1.92±0.1, P<0.01) and tumors (1.5±0.38 vs. 3.8±0.95, P<0.001) in AOM-treated mice, while high-fat diets alone significantly increased aberrant crypts (ACF) (9.3±0.88 vs. 6.3±0.65), and macroscopic tumors (3.8±0.95 vs. 2.0±0.25) compared to mice on a control diet. However, they did not observe if carnitine had any protective effect on intestinal tumors in ApcMin/+ mice.[19]

Roscili *et al.* evaluated the anti-tumor effects of carnitine and curcumin by inducing tumor in mice with carcinogen DMH. They found that carnitine and acyl-carnitine were able to prevent the formation of cancer lesions. They also found that the combination of palmitoyl-carnitine, l-carnitine and acylcarnitine was effective in inhibiting the formation of lesions through all the steps of tumor development and progression: from double ACF, to multiple ACF and to early and late adenomas[29].

Pathological evaluation on late-stage adenomas

Roscili *et al.* evaluated H/E stained sections from gross lesions or whole colons and analyzed tumors from each experimental group. To assess the effect of curcumin and carnitines on the progression of late-stage colon adenomas. They found that there was no macroscopic difference in Adenomas between mice treated with curcumin or carnitines and those in untreated group [29].

Carnitine, apoptosis and cell death

Wenzel *et al.* [26,27], Roy *et al.* [25] and Roscili *et al.* [29] also assessed the effects of carnitine on the cancer cell death and the variables affecting the apoptosis of these cells in various adenocarcinoma cell cultures. Three of these studies found carnitine to have positive effects on both apoptosis and fragmentation DNA in cancerous cells. But in a study by Roscili *et al.*[29], carnitine did not show any significant effect to induce apoptosis in HT-29 cancer cells, except for the curcumin group.

Effects on the activity of caspase-3

In a study by Wenzel *et al.* they provided HT-29 cells with 2 mmol/L of carnitine with palmitoyl-carnitine (PC) and flavones for 24 hours, which significantly increased the activity of the pro-apoptotic protein of Caspase-3 and as a result, an increase in apoptosis was observed in these cells ($p<0.001$). Also, providing carnitine with the same combination just like above but for 36 hours, increased the fragmentation of DNA in these cancer cells up to 90% ($p<0.001$) [26]. In another study by Wenzel *et al.* provided human colonic cell line (NCOL-1) with 2 mmol/L of carnitine with PC. It was found that although this combination has positive effects on the activity of the Caspase-3 and caused an increase of 70 fold in their activity compared to the control group, but has no incremental effect on the apoptosis in these cells ($p <0.001$) [27].

Elimrani *et al.* found that caspases cleave, poly (ADP-ribose) polymerase (PARP), during apoptosis. They also found that although no increment was observed in the level of cleaved PARP in response to carnitine or ALCAR, but butyrate increased this factor [28].

Butyrate and carnitine regulate the activity of BCL proteins in pro-apoptotic and anti-apoptotic pathways

Wenzel *et al.* [27] and Roy *et al.* [25] according to their study they observed that carnitine has positive effects on the increase of apoptosis in cancerous cells. Providing 2 mmol/L carnitine and 100 µmol/L PC for 24 hours to the HT-29 cells resulted in a reduction of anti-apoptotic protein Bcl-XL levels and induction of apoptosis ($P <0.05$). However, the addition of this compounds to the non-transformed NCOL-1 did not affect the levels of Bcl-XL in this cancer cells [27]. Roy *et al.* also found that providing 10 mM carnitine to the Caco-2 cells for 24 hours reduced the expression of the Bcl-xL protein and so increased apoptosis in these cells, the same effect was observed by providing 5 mM and 10 mM butyrate as well. By providing these two supplements together with the same amount to this cell cultures, they observed a further decrease in the Bcl-xL protein levels and the maximum reduction was in the presence of 10 mM carnitine and 2.5 mM butyrate [25].

Two of the most important pro-apoptotic proteins in the cancer cells are Bax and Bak. Roy *et al.* in their study showed that although providing 2.5-10 mM butyrate for 24 hours to Caco-2 cells did not have any significant effect on the levels of these two proteins, but adding 10mM of carnitine to these cells with the same amounts of butyrate, significantly increased the expression of Bax and Bak proteins ($P <0.05$). It should be noted that the levels of Bax protein was increased in the presence of carnitine and depended on the dose of the butyrate. The maximum expression of Bak was observed in the presence of carnitine and 5 mM butyrate [25].

Carnitine and cell death

Incubating caco-2 cells with 10 mM carnitine alone did not significantly affect cell death after 48 hours, but providing 2.5-10 mM butyrate to these cells resulted in a five-fold increment in cell death, in comparison with control cells with a cell death rate of 12.9%, it reached a death rate of 65.7% after 48 hours ($P <0.05$). However, by providing the combination of butyrate and carnitine at the same time, a further increase in cell death was observed compared to when butyrate alone was provided. This shows the positive effect of carnitine on increasing cell death in cancerous cells. Roy *et al.* in their study on Caco-2 cells also found that butyrate increased cell apoptosis, which is the maximal state was 185% ($P <0.05$) And carnitine also caused cell death in cancer cells with a significant increase in apoptosis to 124% compared to control group and the combination of these two induced a significant apoptotic

effect reaching a maximum of 163% after 48 hours (P <0.05) [25].

Elimrani *et al.* investigated the effect of 2 or 3 mM butyrate alone and in combination with 5 mM carnitine or 5 mM acetyl L-carnitine (ALCAR) on the cell death in SW480 cells after 48 hours. They found that cancer cells death was significantly increased with 2 and 3 mM butyrate alone (p <0.01, p <0.05), as with acetyl L-carnitine alone (p <0.05). The carnitine ester (ALCAR) potentiated the effect of butyrate, as cell death increased (p <0.05). However, Addition of carnitine did not significantly affect the cell death in SW480 cells. They also found that 2-3 mM butyrate alone increased apoptosis fourfold after 48 hours, while 5 mM of carnitine and ALCAR alone had no effect. The combination of butyrate and carnitine or ALCAR induced slight apoptotic effects after 48 hours, but it did not show any statistical difference compared with butyrate alone [28].

Inhibiting DNA synthesis and proliferation of cancer cells

Roy *et al.* in their study observed that DNA synthesis was significantly inhibited by providing a variety of 2.5-20 mM butyrate to Caco-2 cells, which in the maximum state reached an inhibitory effect of 52.9% compared with control group. Providing 10 mM carnitine for 48 hours to this cells significantly decreased the proliferation of Caco-2 cells to 89% of the control values. The combination of 10 mM carnitine and 20 mM butyrate showed maximal inhibitory effect with proliferation reduced to 39.8% of the control group (P <0.05) [25].

In a study by Roscili *et al.* HT-29 cells were incubated with increasing concentrations of curcumin extract in the presence or absence of 10 mM carnitine to determine their effect on cell proliferation. They found that all the tested carnitines doses showed the ability to sensitize HT-29 cell to curcumin treatment. Sensitization of All carnitines ranged from 1.28 to 1.89 that suggest these substances have cooperative effects potentially [29].

Elimrani *et al.* in their study measured changes in proteins which are implicated in cell cycle progression, apoptosis and acetylation of histone proteins to investigate the mechanisms of the action of ALCAR on SW480 cells. They found that Butyrate alone induced significant changes in all of the proteins studied, while carnitine and ALCAR showed no significant effect. In most of the colorectal cancer cells, there are mutations of the APC gene that allows nuclear translocation of dephosphorylated β-catenin and upregulation of target genes such as Survivin and cyclin D1. Elimrani *et al.* also observed that dephospho-β-catenin was downregulated and acetylated histone H4 levels were increased by butyrate alone [28].

Carnitine and prostaglandin E2 production via the COX-2 enzyme pathway

Roy *et al.* in their study showed that carnitine may have inhibitory effects on the prostaglandin production in the pathway of cyclooxygenase in Caco-2 cells. This data is useful for decreasing cell inflammation and reducing the probability of cancer. After treating Caco-2 cells with 10 mM carnitine for 48 hours, they observed a significant decrease in the production of prostaglandin in these cells, which reached 85% compared with the control group. They also found such an inhibition by providing 2.5-20 mM butyrate alone for 48 hours, and the maximal effect was 71% versus the control group. They observed maximum decrease with an inhibition rate of PGE2 to 57% of control cells by providing the combination of butyrate and carnitine (P <0.05) [25].

Carnitine and mitochondrial uptake of palmitic acid and producing O^{2-}

In two studies by Wenzel *et al.*[26,27], they found that carnitine increased the production of

superoxide anions (O^{2-}) by enhancing mitochondrial uptake of palmitic acid and so induced apoptosis in cancer cells. This indicates the positive role of carnitine on the cancer cell death. In a study by Wenzel *et al.* provision of 100 µM palmitoylcarnitine to HT-29 cells after 4 hours increased the mitochondrial uptake of palmitic acid and promotes flavone-induced apoptosis more than doubled in these cells. This effect was also observed by providing 150 µM flavone for 4 hours to these cells. In this study, the combination of carnitine and flavone did not significantly increase the uptake of fluorescent palmitic acid into mitochondria. While each of carnitine and flavone alone increased the transfer of palmitic acid into mitochondria ($p<0.05$) [26].

In another study, Wenzel *et al.* Found that carnitine significantly increases the mitochondrial uptake of fluorescent palmitic acid analog. After exposing HT-29 cells with 100 µmol/L of PAFA plus 2 mmol/L of carnitine for 4 hours, they observed that mitochondrial uptake of palmitic acid fluorescent palmitic acid analog increased by a highly potent, while it was low generally. They provided similar carnitine and PAFA to NCOL-1 cells in the same condition and found that although these cells displayed a higher mitochondrial uptake of fatty acid analog intrinsically, but palmitic acid influx wasn't increased significantly under the influence of carnitine ($P <0.05$) [27].

Discussion:

In this study, we reviewed several articles, which have investigated the relationship between l-carnitine and colorectal cancer. In this study, factors affecting the cancer cell death such as the concentration of pro-apoptotic proteins caspase-3, bax, bak and the anti-apoptotic protein Bcl-Xl as well as the level of superoxide Anion (O^{2-}) produced by palmitic acid oxidation in mitochondria and the amount of prostaglandin produced via cyclooxygenase pathway has been studied under the influence of carnitine.

Aberrant Crypt Foci, Crypt Multiplicity, and Macroscopic Tumors

A study by Dionne *et al.*[19] on the effects of carnitine consumption and high-fat diets on the development of precancerous lesions and progression toward macroscopic tumors that was performed on APC and AOM mice in 2012, expressed that, although temporal trends and migrants studies showed that the cause of colorectal cancer was predominantly environmental and could potentially be modified, but numerous prospective and case-control studies have indicated an association between diet and colon cancer. Dionne *et al.* in their study observed that although high-fat diet increased tumors and lesions in the colon and intestine of these mice, but the addition of carnitine to the diet inhibited colonic tumor development in AOM-treated animals, but it did not have a positive effect on genetic APC Min/+ model of colorectal cancer.

Milk fat contains proven anti-cancer compounds, such as linoleic acid with conjugated bands, sphingolipids and butyric acid, which may have an inhibitory effect on the progression of small polyps to larger ones. Colon carcinogenesis is a multistep process involving initiation and progression. Although Western diet can induce tumors at low incidence in long time, high fat intake can aid colon, especially during its progression phase. Various studies have shown that a high-fat diet promotes tumor progression by increasing cell proliferation or decreasing the amount of apoptosis occurs in initiated colon cancer cells. A number of mechanisms by which high-fat diet affect tumor progression including oxidative stress; increased levels of COX-2, PGE2 and diacylglycerol; activation of Ras,

PKC, and ODC; as well as upregulation of inflammatory cytokines; and alterations in the gut microbiome.

Carnitine is an anti-free radical factor and inhibitor of lipid peroxidation. Its ability to protect tissues from oxidative damage is well known. The inhibition of hepatocarcinogenesis in rats by l-carnitine is due to the reduction of oxidative stress, decreased free fatty acids levels, increased ROS production, and prevention of mitochondria damages. AOM can cause to induce ACF formation in the colon by creating oxidative stress that results in the destruction of DNA and mutations in cancer-related genes. The fatty acid intermediates accumulation may disrupt mitochondrial function and contribute to ROS generation and oxidative stress. It has been shown that carnitine can lower the levels of matrix acyl-coA and prevent excessive mitochondrial ROS.

Carnitine, apoptosis and cell death (influenced by regulatory proteins and mitochondrial O2- production)

Studies by Wenzel *et al.* [26,27], Roy *et al.* [25] investigated the relationship between carnitine and the death of cancer cells and the variables affecting these cell's apoptosis in different colorectal adenocarcinoma cell cultures. All the three studies found positive effects of carnitine on increasing apoptosis and DNA fragmentation in cancerous cells. When the transport of lactate and pyruvate into mitochondria increases, the available substrate for acetyl-CoA generation for oxidation metabolism also increases; so the production of superoxide Anion (O2) in the mitochondria is rapidly accelerated and apoptosis occurs.

Wenzel *et al.* in their study examined whether the increased beta-oxidation of fatty acids in mitochondria could increase apoptosis in colon cancer cells. In their research, they found that providing a combination of PC and carnitine for the HT-29 human colon cancer cells induced apoptosis in these cancer cells. However, there was no indication of an increase in apoptosis in a non-transformed colonic epithelial cell line NCOL-1, and since the production of mitochondrial O^{2-} was also lower in these cells, it seems that these cells have a higher antioxidant capacity than transformed cells. They found that no PC and carnitine alone were able to call for apoptosis in HT-29 cells. In the absence of external fatty acids, carnitine seems to be unaffected; this may be due to the lack of or insufficient endogenous production of FFA for uptake into mitochondria. They also showed that human colon cancer cells had a lower free carnitine level.

It has also been shown that the ratio of free carnitine concentration to carnitine esters in cancer patients has changed in comparison with the control group, which could provide a basis for suggesting the presence of a cancer-related metabolic disorder in relation with the availability of carnitine. Carnitine can also enhance glucose oxidation by increasing mitochondrial acylcarnitine uptake and providing a substrate for β-oxidation, as during this process acetyl-CoA is separated into its constructive units and via carnitine/acetyl-transferase delivering acetyl groups to the cytosol and free CoA to the matrix. The removal of the mitochondrial acetyl-COA which is carnitine-dependent leads to releases the inhibition of pyruvate dehydrogenase and enables the use of pyruvate with mitochondrial free CoA, which increases oxidation. They showed that the combination of PC and carnitine could result in a similar reduction in levels of this protein as a key regulatory protein in the apoptotic process.

In murine B-lymphoma cell lines, it has been shown that palmitate elicits apoptosis by increasing the synthesis of ceramide sphingolipid which is a known apoptosis inducer. However, this ceramide synthesis does

not play a role for apoptosis, which is initiated by pc and carnitine in HT-29 cells [27].

Roy *et al.* in their study suggested that, in fact, this is butyrate, which can prevent the growth and proliferation of neoplasms and significantly decrease the proliferation of Caco-2 cells by blocking the growth and proliferation of cells in the phase G1 and G2/M by inhibiting regulatory proteins. Carnitine also had an inhibitory effect on the proliferation and growth of Caco-2 colon cancer cells in a dose-dependent manner; this is maybe due to its ability to inhibit oxidative damage or inducing oxidative effects. This inhibitory effect is further enhanced by the presence of the combination of both carnitine and butyrate because of short half-life and rapidly metabolized of butyrate by epithelial cells, it does not reach the maintenance of effective concentrations in the colon mucosa to have anti-carcinogenic benefits, but carnitine can increase its half-life and so increases its effects. It is also suggested that butyrate irregulates the expression of carcinogenic genes by hyper-acetylating histone and hyper-methylated DNA.

Roy *et al.* in their study also observed that the expression of anti-apoptotic protein Bcl-xl changed in Caco-2 cells treated with butyrate. When treating with carnitine, as no changes in the regulation of pro-apoptotic proteins of Bak and Bax was shown, there was low apoptotic effects induced. But in response to combination of butyrate and carnitine, changes in the amount of Bak and Bax proteins were also observed. These results significantly altered the ratio of Bax to Bcl-Xl and Bak to Bcl-Xl for inducing apoptosis[25].

Wenzel *et al.* stated in their study that one of the most important features of the Cancer cells metabolism is an adaptation to the special local environment. Even being under hypoxic conditions and adaptation to low oxygen tension, will be a crucial step toward tumor progression. The main important thing about tumor-specific metabolic alterations is that some genetic alterations, which affect metabolic pathways enzymes, promote tumor cell growth directly. The common feature of most tumors is the anaerobic use of glucose as a source of energy through glycolysis, resulting in low levels of fatty acids oxidation, which causes low energy yield due to the lack of complete substrate oxidation. This adaptation minimizes ROS production in rapidly growing cancer cells and protects DNA and proteins to be damaged by oxygen radicals, which produced during oxidative phosphorylation [26]. In HT-29 human colon cancer cells, impaired transport of pyruvate and lactate as the final products of glycolysis into mitochondria prevents substrate oxidation. Exposing these cells to flavone increased pyruvate uptake into mitochondria with a consequent increase in ATP production. O2- increases in mitochondria during apoptosis process, and SO when there is enhancement in fatty acids uptake into mitochondria, these cells exhibited significant increase in mitochondrial O^{2-} production and apoptosis.

Wenzel *et al.* Showed that exposing HT-29 cells to flavone increases the levels of free carnitine in their mitochondria, which in turn increases PC uptake into mitochondria and its oxidation. Their data also suggest that flavone not only activates mono-carboxylate transporters of the mitochondrial membrane, but also free carnitine transporters such as organic cation transporters. Interestingly, while the cytosolic levels of free carnitine were essentially the same in both cell lines of HT-29 and NCOL-1, flavone increased mitochondrial free carnitine levels in HT-29 cells till reach to the same levels as in NCOL-1 cells mitochondria. Overall, they found that although mitochondrial fatty acids uptake in NCOL-1 cells is not dependent on providing external carnitine, the data from HT-29 cells strongly suggests that increased mitochondrial uptake of

carnitine which enhances counter-transport with acyl-carnitines via the acyl-carnitine/carnitine translocase into the mitochondria limits beta-oxidation rate in colon cancer cells, but not in non-transformed cells.

In both HT-29 and NCOL-1 cell lines, fatty acids oxidation inevitably causes mitochondrial O^{2-} generation. Although this generation can be compensated in NCOL-1 cells due to their higher antioxidative capacity, when the antioxidative capacity of NCOL-1 cells decreases, substrate oxidation increases and induces apoptosis. Wenzel *et al.* stated in their study that in addition to accelerating the import of fatty acids into mitochondria (by increasing the amount of free carnitine), flavone can also increase the release of free fatty acids from endogenous triglycerides, just as it was suggested by the positive effects of carnitine to promoting apoptosis in cells treated with flavone.

However, providing external fatty acids to inducing apoptosis in cells that were exposed with flavone was much more effective than providing carnitine for these cells. This suggests that the availability of free fatty acids for beta-oxidation limits the amount of acetyl-CoA production for oxidative metabolism. Acyl-carnitines implicate in the acetyl-coenzyme A regulation increasing ratio of acetyl-CoA to CoA[30,31]. Acyl-carnitines also provides acetyl group for nuclear protein acetylation[32].

Roscili *et al.* used three form of carnitine; L-carnitine (LC), Acetyl-L-carnitine (ALC), propionyl-L-carnitine (PLC) in their study and found that carnitines potentiated anti-proliferative effects of curcumin which increased apoptosis in HT-29 cells incubated with the carnitine-curcumin mixture. They also observed in the Annexin v assay that live cells were reduced and also dead cells population was increased in parallel by adding carnitines to curcumin treatment. In the vitro apoptosis assay, they found that LC had a great potential to sensitize colon cancer cells to curcumin treatment. In different stages of induced colon lesions, single carnitine and acyl-carnitines had equal or exceed effect than curcumin [29].

Carnitine and mitochondrial uptake of palmitic acid and producing O2-

Roy *et al.* in their studies found that apoptotic cell death resulting from the combination of butyrate and carnitine is companied with inhibition of the pro-oncogenic COX-2 enzyme. They also found that COX-2 protein and PGE2 levels were equally affected by treatment. They stated that it's been proven among the most studies that PGE2 can induce cell proliferation in colon cells and inhibit apoptosis in them. Several mechanisms have also been discovered for this case. By providing the appropriate amount of PGE2 to Caco-2 cells they observed that when this cell culture was treated with butyrate, the PGE2 levels decreased and also when it was treated with carnitine, PGE2 synthesis was significantly reduced as well. But when these cancer cells were treated with combination of carnitine and butyrate, the PGE2 production decreased much better [25].

Table 1: Characteristics of included publications

References	Type of study	Animals or cell cultures	Duration	Treatment	Results
Dionne *et al.*,2012 [19]	Experimental Parallel design	AOM mice	40 Weeks	Control gp: AIN-93 2nd gp: Safflower oil 3rd gp : 15 % Butterfat 4th gp :15 %Butterfat + 0.08% Carnitine	AOM-exposed mice on a high butterfat diet had significantly increased aberrant crypts, and macroscopic tumors compared to mice on a control diet. In AOM mice fed the high butterfat diet, carnitine supplementation inhibited ACF, crypt multiplicity and tumors. Carnitine supplementation resulted in significantly increased tissue carnitine and acyl-carnitine levels. Carnitine inhibited the development of precancerous lesions and macroscopic colonic tumors in AOM-treated mice. However, carnitine did not exert protective effects on intestinal tumors in ApcMin/+ mice.
Elimrani *et al.*,2015 [28]	Interventional Parallel design	SW480 Cells Caco-2 Cells	48 Hours	2 and 3 mM butyrate 5 mM carnitine 5 Mm ALCAR	Cells treated with the combination of butyrate (3 mM) with ALCAR exhibited increased mortality. The addition of carnitine or ALCAR also increased butyrate-induced apoptosis. Butyrate increased levels of cyclin D1, p21, and PARP p86, but decreased Bcl-XL and survivin levels. Butyrate also downregulated dephospho-β-catenin and increased acetylated histone H4 levels. Butyrate and carnitine decreased survivin levels by ≥25%. ALCAR independently induced a 20% decrease in p21. These results demonstrate that butyrate and ALCAR are potentially beneficial anti-carcinogenic nutrients that inhibit colon cancer cell survival *in vitro*. The combination of both agents may have superior anti-carcinogenic properties than butyrate alone.
Roscili *et al.*,2013 33	Experimental -Parallel design	DMH treated mice & HT-29 Cells	20 Weeks & 24, 48 ,72 Hours	10mM carnitine (LC, ALC, PLC) 10 mM curcumin	It was observed that carnitine and acyl-carnitines had same, if not higher, efficacy than curcumin alone in inhibiting the formation of neoplastic lesions induced by DMH treatment. Interestingly, the combination of curcumin and acetyl-L-carnitine was able to fully inhibit the development of advanced adenoma lesions. The data unveil the antitumor effects of carnitines and warrant additional studies to further support the adoption of carnitines as cancer chemo-preventative agents.

References	Type of study	Animals or cell cultures	Duration	Treatment	Results
Roy *et al.*,2009 [25]	Experimental -Parallel design	Caco-2 Cells	24, 48 Hours	2.5–20 mM butyrate 10 mM carnitine	Butyrate and carnitine inhibited Caco-2 cell proliferation (P< 0.05) and induced apoptosis. Prostaglandin E2 production was decreased in treated Caco-2 cells. At the molecular level, the expression of pro-apoptotic Bax and Bak proteins were increased in cells incubated with butyrate and carnitine, whereas expression of anti-apoptotic Bcl-xL was decreased. Cyclo-oxygenase-2 expression was decreased in cells incubated with butyrate and carnitine.
Wenzel *et al.*,2005(Oct) [26]	Experimental -Parallel design	HT-29 Cells	4, 24, 36 Hours	2 mM carnitine 100 µM palmitoyl - carnitine 150 µM flavone	Here they show that flavone-induced apoptosis is increased more than twofold in the presence of palmitoyl carnitine due to increased mitochondrial fatty acid transport and the subsequent metabolic generation of O^{2-}.in mitochondria is the initiating factor for the execution of apoptosis.
Wenzel *et al.*,2005(March) [27]	Experimental -Parallel design	NCOL-1 Cells HT-29 Cells	4, 6,24,36 Hours	2 mmol/L carnitine 100 µmol/L palmitoyl - carnitine 100 µmol/L fluorescent palmitic acid analog 10 µmol/L benzoquinone 1 µmol/L fumonisin	They investigated whether an increased supply of fatty acids for mitochondrial β-oxidation is also able to induce the generation of O^{2-} that then leads to apoptosis. To assess whether these metabolic effects on the execution of apoptosis are specific for colonic tumor cells, the non-transformed human colonocyte cell line NCOL-1 (4) served as a control

Conclusion:

Data from the investigated studies shows that carnitine may have potential anticancer effects and inhibits the progression of macroscopic and pre-cancerous tumors and prevents the growth and proliferation of cancer cells. Data also suggest that L-carnitine may have synergistic effects with butyrate in the prevention and treatment of precancerous lesions.

References:

1 Ferlay, J. *et al.* Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International journal of cancer* 136, E359-E386 (2015).

2 WHO, global health observatory in 2015. available at *www.who.int*.

3 WHO, Noncommunicable disease (NCD) country profiles (Iran) in 2014. available at www.who.int

4 Fateh, S. & AMINI, M. An epidemiologic study of colorectal cancer in Arak during 1994-2004. 2(16), 11-17 (2008).

5 Corman, M. L. Colon, and Rectal Surgery. *4th ed. New York: Lippincott company*, 625-762 (1999).

6 WHO, IARC GLOBOCAN, Cancer Incidence and Mortality Worldwide in 2008. available at: http://globocan.iarc.fr/.

7 Fauci, A. *Harrison's Gastroenterology and Hepatology*. (McGraw-Hill Professional, 2010).

8 World Health Organization. Cancer Country Profiles (Iran) in 2014. Available at *www.who.int*.

9 Azizi, F., Hatami, H. & Janghorbani, M. Epidemiology and control of prevalent

diseases in Iran. *Tehran. Khosravi Publication*, 265-279 (2004).

10 WHO, programmes, cancer. available at www.who.int.

11 Jansman, F. & Postma, M. Costs of chemotherapy in the treatment of colorectal cancer. *The European Journal of Health Economics* 7, 145-146 (2006).

12 Byford, S., Torgerson, D. J. & Raftery, J. Cost of illness studies. *BMJ: British Medical Journal* 320, 1335 (2000).

13 Robbins, S., Cotran, R. & Kumar, V. Robbins pathologic basis of disease 6th ed. *Philadelphia, Pa: Saunders*, 802-808 (1999).

14 Becker, N. Epidemiology of colorectal cancer. *Der Radiologe* 43, 98-104 (2003).

15 MOVAHEDI, M., BISHOP, T. & BARRETT, J. Association between alcohol, dietary factors and subsites of colorectal cancer: an ecological study. 8(1), 53-60 (2006).

16 Han-Shiang, C. Curative resection of colorectal adenocarcinoma: multivariate analysis of 5-year follow-up. *World journal of surgery* 23, 1301-1306 (1999).

17 Biochemical mechanisms of colorectal cancer. Research in Medical Science 31 (2008)

18 Reuter, S. E. & Evans, A. M. Carnitine and acylcarnitines. *Clinical pharmacokinetics* 51, 553-572 (2012).

19 Dionne, S. *et al.* Studies on the chemopreventive effect of carnitine on tumorigenesis in vivo, using two experimental murine models of colon cancer. *Nutrition and cancer* 64, 1279-1287, doi:10.1080/01635581.2012.722247 (2012).

20 Penn, D., Dolderer, M. & Schmidt-Sommerfeld, E. Carnitine concentrations in the milk of different species and infant formulas. *Neonatology* 52, 70-79 (1987).

21 İzgüt-Uysal, V. N. *et al.* Effect of chronic stress and L-carnitine on rat stomach. *The Journal of Physiological Sciences* 57, 187-192 (2007).

22 Steiber, A., Kerner, J. & Hoppel, C. L. Carnitine: a nutritional, biosynthetic, and functional perspective. *Molecular aspects of medicine* 25, 455-473 (2004).

23 Gülçin, İ. Antioxidant and antiradical activities of L-carnitine. *Life sciences* 78, 803-811 (2006).

24 Flanagan, J. L., Simmons, P. A., Vehige, J., Willcox, M. D. & Garrett, Q. Role of carnitine in disease. *Nutrition & metabolism* 7, 30 (2010).

25 Roy, M. J. *et al.* In vitro studies on the inhibition of colon cancer by butyrate and carnitine. *Nutrition (Burbank, Los Angeles County, Calif.)* 25, 1193-1201, doi:10.1016/j.nut.2009.04.008 (2009).

26 Wenzel, U., Nickel, A. & Daniel, H. Increased mitochondrial palmitoylcarnitine/carnitine countertransport by flavone causes oxidative stress and apoptosis in colon cancer cells. *Cellular and molecular life sciences : CMLS* 62, 3100-3105, doi:10.1007/s00018-005-5378-7 (2005).

27 Wenzel, U., Nickel, A. & Daniel, H. Increased carnitine-dependent fatty acid uptake into mitochondria of human colon cancer cells induces apoptosis. *The Journal of Nutrition* 135, 1510-1514 (2005).

28 Elimrani, I. *et al.* Acetylcarnitine potentiates the anticarcinogenic effects of butyrate on SW480 colon cancer cells. *International journal of oncology* 47, 755-763, doi:10.3892/ijo.2015.3029 (2015).

29 Roscilli, G. *et al.* Carnitines slow down tumor development of colon cancer in the DMH-chemical carcinogenesis mouse model. *Journal of cellular biochemistry* 114, 1665-1673, doi:10.1002/jcb.24508 (2013).

30 Steiber, A., Kerner, J. & Hoppel, C. L. Carnitine: a nutritional, biosynthetic, and functional perspective. *Molecular aspects of medicine* 25, 455-473 (2004).

31 Calabrese, V., Stella, A. M. G., Calvani, M. & Butterfield, D. A. Acetylcarnitine and cellular stress response: roles in nutritional redox homeostasis and regulation of longevity genes. *The Journal of nutritional biochemistry* 17, 73-88 (2006).

32 Madiraju, P., Pande, S. V., Prentki, M. & Madiraju, S. M. Mitochondrial acetylcarnitine provides acetyl groups for nuclear histone acetylation. *Epigenetics* 4, 399-403 (2009).

33 Roscilli, G. *et al.* Carnitines slow down tumor development of colon cancer in the DMH-chemical carcinogenesis mouse model. *Journal of cellular biochemistry* 114, 1665-1673 (2013).