

## **Vitamin C in human health and disease is still a mystery? An overview**

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## Abstract

Ascorbic acid is one of the important water soluble vitamins. Its deficiency causes scurvy which is a rare disease in recent times. Most plants and animals synthesize ascorbic acid for their own requirement. However, guinea pigs, fruit eating bats, apes and humans cannot synthesize ascorbic acid due to lack of an enzyme gulonolactone oxidase. Hence ascorbic acid has to be supplemented mainly through fruits, vegetables and synthetic tablets. The current recommended daily allowance (RDA) for ascorbic acid ranges between 100 - 120 mg/per day for adults. Ascorbic acid is essential for many physiological functions including synthesis of collagen, carnitine and neurotransmitters; facilitates absorption of iron, helps in metabolism of cholesterol. In addition, ascorbic acid is a potent chain breaking water soluble antioxidant and can neutralize a wide range of reactive oxygen and nitrogen species in the body. It acts as a co-antioxidant in regeneration of alpha-tocopherol and also prevents its pro-oxidant activity. Although ascorbic acid has strong antioxidant properties, it also has pro-oxidant effects in the presence of free transition metals. Many health benefits have been attributed to ascorbic acid such as anti-atherogenic, antioxidant, anti-carcinogenic, immunobooster during cold attacks. However, lately the health benefits of ascorbic acid have been the subject of debate and controversies viz., Danger of mega doses of ascorbic acid? Does ascorbic acid act as a pro-oxidant? Does ascorbic acid cause cancer? Ascorbic acid may interfere with cancer therapy?. However, the Panel on dietary antioxidants and related compounds stated that the *in vivo* data do not clearly show a relationship between excess ascorbic acid intake and kidney stone formation, pro-oxidant effects, excess iron absorption. A number of clinical and epidemiological studies on anti-carcinogenic effects of ascorbic acid in humans did not show any conclusive beneficial effects on various types of cancer except gastric cancer. Recently, a few derivatives of ascorbic acid were tested on cancer cells, among them ascorbic acid esters showed potential activity compared to ascorbic acid.

Ascorbyl stearate was found to inhibit proliferation of human cancer cells by interfering with cell cycle progression, induced apoptosis by modulation of signal transduction pathways. However, more mechanistic human *in vivo* studies are needed to understand and elucidate the molecular mechanism underlying the anti-carcinogenic property of ascorbic acid. Thus, though ascorbic acid was discovered in 17<sup>th</sup> century, the exact role of this vitamin/nutraceutical in human health and disease is still a mystery in view of many health beneficial claims and their controversies.

### **Historical perspective**

The sea voyager/sailors developed a peculiar disease called scurvy when they were on sea. This was found to be due to eating non-perishable items and lack of fresh fruits and vegetables in their diet. A British naval Physician, Lind [1] documented that there was some substance in citrus fruits that can cure scurvy. He developed a method to concentrate and preserve citrus juice for use by sailors. British Navy was given a daily ration of lime or lemon juice to overcome ascorbic acid deficiency. Ascorbic acid was first isolated from natural sources and structurally characterized by Szent-Gyorgyi, Waugh and King [2,3]. This vitamin was first synthesized by Haworth and Hirst [4]. Currently ascorbic acid is the most widely used vitamin supplement throughout the world.

### **Chemistry of ascorbic acid**

L-ascorbic acid ( $C_6H_8O_6$ ) is the trivial name of Vitamin C. The chemical name is 2-oxo-L-threo-hexono-1,4-lactone-2,3 enediol. L-ascorbic and dehydroascorbic acid are the major dietary forms of vitamin C [5]. Ascorbyl palmitate is used in commercial antioxidant preparations. All commercial forms of ascorbic acid except ascorbyl palmitate are soluble in water. L-ascorbic acid and its fatty acid esters are used as food additives, antioxidants, browning inhibitors, reducing agents, flavor stabilizers, dough modifiers and color stabilizers. Ascorbyl

palmitate has been used for its greater lipid solubility in antioxidant preparations. In foods, pH influences the stability of ascorbic acid. It exhibits maximal stability between pH 4 and 6 [5]. Cooking losses of ascorbic acid depend on degree of heating, surface area exposed to water, oxygen, pH and presence of transition metals.

### **Catabolism of Ascorbic acid**

Ascorbic acid present in foods is readily available and easily absorbed by active transport in the intestine [6]. Most of it (80 -90%) will be absorbed when the intake is up to 100 mg/day, whereas at higher levels of intake (500 mg/day) the efficiency of absorption of ascorbic acid rapidly declines. Ascorbic acid is sensitive to air, light, heat and easily destroyed by prolonged storage and over processing of food.

Ascorbic acid being a water soluble compound is easily absorbed but it is not stored in the body. The average adult has a body pool of 1.2 -2.0g of ascorbic acid that may be maintained with 75 mg/d of ascorbic acid. About 140 mg/d of ascorbic acid will saturate the total body pool of vitamin C [7]. The average half life of ascorbic acid in adult human is about 10 -20 days, with a turn over of 1 mg/kg body and a body pool of 22 mg/kg at plasma ascorbate concentration of 50  $\mu\text{mol/L}$  [8,9]. Hence ascorbic acid has to regularly supplemented through diet or tablets to maintain ascorbic acid pool in the body.

The major metabolites of ascorbic acid in human are dehydroascorbic acid, 2,3-diketogulonic acid and oxalic acid (Fig 1). The main route of elimination of ascorbic acid and its metabolites is through urine. It is excreted unchanged when high doses of ascorbic acid are consumed. Ascorbic acid is generally non-toxic but at high doses (2 -6 g/day) it can cause gastrointestinal disturbances or diarrhea [10,11]. The side effects are generally not serious and can be easily

reversed by reducing intake of ascorbic acid. Furthermore, there is no consistent and compelling data on serious health effects of vitamin C in humans [11].

### **Sources of Ascorbic acid**

Ascorbic acid is widely distributed in fresh fruits and vegetables. It is present in fruits like orange, lemons, grapefruit, watermelon, papaya, strawberries, cantaloupe, mango, pineapple, raspberries and cherries. It is also found in green leafy vegetables, tomatoes, broccoli, green and red peppers, cauliflower and cabbage.

Most of the plants and animals synthesize ascorbic acid from D -glucose or D -galactose. A majority of animals produce relatively high levels of ascorbic acid from glucose in liver (Fig 2).

However, guinea pigs, fruit eating bats, apes and humans can not synthesize ascorbic acid due to the absence of the enzyme L -gulonolactone oxidase. Hence, in humans ascorbic acid has to be supplemented through food and/or as tablets.

Ascorbic acid is a labile molecule, it may be lost from foods during cooking/processing even though it has the ability to preserve foods by virtue of its reducing property. Synthetic ascorbic acid is available in a wide variety of supplements viz., tablets, capsules, chewable tablets, crystalline powder, effervescent tablets and liquid form. Buffered ascorbic acid and a dehydrated form of ascorbic acid as ascorbyl palmitate is also available commercially. Both natural and synthetic ascorbic acid are chemically identical and there are no known differences in their biological activities or bio -availability.

A deficiency of ascorbic acid leads to scurvy. It is characterized by spongy swollen bleeding gums, dry skin, open sores on the skin, fatigue, impaired wound

healing and depression [13]. Scurvy is of rare occurrence nowadays due to adequate intake of ascorbic acid through fresh vegetables and fruits and or supplementation as tablets.

### **Dietary recommendations of Ascorbic acid**

The new average daily intake level that is sufficient to meet the nutritional requirement of ascorbic acid or recommended dietary allowances (RDA) for adults (>19 yr) are 90 mg/day for men and 75 mg/day for women [14]. Consumption of 100 mg/day of ascorbic acid is found to be sufficient to saturate the body pools (neutrophils, leukocytes and other tissues) in healthy individuals. Based on clinical and epidemiological studies it has been suggested that a dietary intake of 100 mg/day of ascorbic acid is associated with reduced incidence of mortality from heart diseases, stroke and cancer [15]. However, stress, smoking, alcoholism, fever, viral infections cause a rapid decline in blood levels of ascorbic acid.

Smoking is known to increase the metabolic turnover of ascorbic acid due to its oxidation by free radicals and reactive oxygen species generated by cigarette smoking [16]. It has been suggested that a daily intake of at least 140 mg/day is required for smokers to maintain a total body pool similar to that of non-smokers consuming 100 mg/day [17]. Based on latest literature reports, it has been recommended that the RDA for ascorbic acid should be 100-120 mg/day to maintain cellular saturation and optimum risk reduction of heart disease, stroke and cancer in healthy individuals [18]. There is no scientific evidence to show that even very large doses of vitamin C are toxic or exert serious adverse health effects [11,19]. Furthermore, the panel on dietary antioxidants and related compounds suggested that *in vivo* data do not clearly show a relationship between excess vitamin C intake and kidney stone formation, pro-oxidant effects, excess iron absorption [20].

## Physiological functions of Ascorbic acid

The physiological functions of ascorbic acid are largely dependent on the oxidation-reduction properties of this vitamin. L-ascorbic acid is a co-factor for hydroxylases and monooxygenase enzymes involved in the synthesis of collagen, carnitine and neurotransmitters [21]. Ascorbic acid accelerates hydroxylation reactions by maintaining the active center of metal ions in a reduced state for optimal activity of enzymes hydroxylase and oxygenase.

Ascorbic acid plays an important role in the maintenance of collagen which represents about one third of the total body protein. It constitutes the principal protein of skin, bones, teeth, cartilage, tendons, blood vessels, heart valves, intervertebral discs, cornea and eye lens. Ascorbic acid is essential to maintain the enzyme prolyl and lysyl hydroxylase in an active form. The hydroxylation of proline and lysine is carried out by the enzyme prolyl hydroxylase using ascorbic acid as co-factor. Ascorbic acid deficiency results in reduced hydroxylation of proline and lysine, thus affecting collagen synthesis.

Ascorbic acid is essential for the synthesis of muscle carnitine ( $\beta$ -hydroxybutyric acid). [22]. Carnitine is required for transport and transfer of fatty acids into mitochondria where it can be used for energy production. Ascorbic acid acts as co-factor for hydroxylations involved in carnitine synthesis. Further, ascorbic acid acts as co-factor for the enzyme dopamine  $\beta$ -hydroxylase, which catalyzes the conversion of neurotransmitter dopamine to norepinephrine. Thus ascorbic acid is essential for synthesis of catecholamines. In addition, ascorbic acid catalyzes other enzymatic reactions involving amidation necessary for maximal activity of hormones oxytocin, vasopressin, cholecystokinin and  $\alpha$ -melanotropin [23].

Ascorbic acid is also necessary for the transformation of cholesterol to bile acids as it modulates the microsomal  $\alpha$ -hydroxylation, the rate limiting reaction of

cholesterol catabolism in liver. In ascorbic acid deficiency, this reaction becomes slowed down thus, resulting in an accumulation of cholesterol in liver, hypercholesterolemia, formation of cholesterol gallstones etc. [24].

### **Ascorbic acid and iron**

Ascorbic acid is known to enhance the availability and absorption of iron from non-heme iron sources [25]. Ascorbic acid supplementation is found to facilitate the dietary absorption of iron. The reduction of iron by ascorbic acid has been suggested to increase dietary absorption of non-heme iron [26]. It is well known that in the presence of redox-active iron, ascorbic acid acts as a pro-oxidant *in vitro* and might contribute to the formation of hydroxyl radical, which eventually may lead to lipid, DNA or protein oxidation [27]. Thus, ascorbic acid supplementation in individuals with high iron and/or bleomycin-detectable iron (BDI) in some preterm infants could be deleterious because it may cause oxidative damage to biomolecules [28,29,30,31]. However, no pro-oxidant effect was observed on ascorbic acid supplementation on DNA damage in presence or absence of iron [32].

### **Ascorbic acid in health and disease**

#### **Ascorbic acid and common cold**

The most widely known health beneficial effect of ascorbic acid is for the prevention or relief of common cold. Pauling [33] suggested that ingestion of 1–2 g of ascorbic acid effectively prevents/ameliorates common cold. The role of oral vitamin C in the prevention and treatment of colds remains controversial despite many controlled trials. Several clinical trials with varying doses of ascorbic acid showed that ascorbic acid does not have significant prophylactic effect, but reduced the severity and duration of symptoms of cold during the period of infection. Randomized and non-randomized trials on vitamin C to prevent or treat

the common cold showed that consumption of ascorbic acid as high as 1.0g/day for several winter months, had no consistent beneficial effect on the incidence of common cold. For both preventive and therapeutic trials, there was a consistent beneficial but generally modest therapeutic effect on duration of cold symptoms. There was no clear indication of the relative benefits of different regimes of vitamin C doses. However, in trials that tested vitamin C after cold symptoms occurred, there was some evidence of greater benefits with large doses than with lower doses [34].

There has been a long -standing debate concerning the role of ascorbic acid in boosting immunity during cold infections. Ascorbic acid has been shown to stimulate immune system by enhancing T -cell proliferation in response to infection. These cells are capable of lysing infected targets by producing large quantities of cytokines and by helping B cells to synthesize immunoglobulins to control inflammatory reactions. Further, it has been shown that ascorbic acid blocks pathways that lead to apoptosis of T -cells and thus stimulate or maintain T cell proliferation to attack the infection. This mechanism has been proposed for the enhanced immune response observed after administration of vitamin C during cold infections [35].

### **Ascorbic acid and wound healing**

Ascorbic acid plays a critical role in wound repair and healing/regeneration process as it stimulates collagen synthesis. Adequate supplies of ascorbic acid are necessary for normal healing processes especially for post -operative patients. It has been suggested that there will be rapid utilization of ascorbic acid for the synthesis of collagen at the site of wound/ burns during post -operative period [36]. Hence, administration of 500 mg to 1.0 g/day of ascorbic acid are recommended to accelerate the healing process [8].

## Ascorbic acid and atherosclerosis

Lipid peroxidation and oxidative modification of low density lipoproteins (LDL) are implicated in development of atherosclerosis [37]. Vitamin C protects against oxidation of isolated LDL by different types of oxidative stress, including metal ion dependent and independent processes [38]. Addition of iron to plasma devoid of ascorbic acid resulted in lipid peroxidation, whereas endogenous and exogenous ascorbic acid was found to inhibit the lipid oxidation in iron-overloaded human plasma [39]. Similarly, when ascorbic acid was added to human serum supplemented with  $\text{Cu}^{2+}$ , antioxidant activity rather than pro-oxidant effects were observed [40].

Ascorbic acid is known to prevent the oxidation of LDL primarily by scavenging the free radicals and other reactive oxygen species in the aqueous milieu [41]. In addition, *in vitro* studies have shown that physiological concentrations of ascorbic acid strongly inhibit LDL oxidation by vascular endothelial cells [42]. Adhesion of leukocytes to the endothelium is an important step in initiating atherosclerosis. *In vivo* studies have demonstrated that ascorbic acid inhibits leukocyte-endothelial cell interactions induced by cigarette smoke [43,44] or oxidized LDL [45]. Further, lipophilic derivatives of ascorbic acid showed protective effect on lipid peroxide induced endothelial injury [46].

A number of studies have been carried out in humans to determine the protective effect of ascorbic acid supplementation (500 - 100 mg/day) on *in vivo* and *ex vivo* lipid peroxidation in healthy individuals and smokers. The findings are inconclusive as ascorbic acid supplementation showed a reduction or no change in lipid peroxidation products [10,47 - 50]. In this context, it is important to note that during *ex vivo* LDL oxidation studies, water soluble ascorbic acid is removed during initial LDL isolation step itself. Therefore, no change in *ex vivo* would be expected [15]. Overall, both *in vitro* and *in vivo* experiments showed that ascorbic acid protects isolated LDL and plasma lipid peroxidation induced by

various radical or oxidant generating systems. However, a recent report demonstrated that large doses of exogenous iron (200mg) and ascorbic acid (75 mg) promoted the release of iron from iron binding proteins and also enhanced *in vitro* lipid peroxidation in serum of guinea pigs. This finding supports the hypothesis that high intake of iron along with ascorbic acid could increase *in vivo* lipid peroxidation of LDL and therefore could increase risk of atherosclerosis [51]. However, Chen et al., [52] demonstrated that ascorbic acid acts as an antioxidant towards lipid even in presence of iron overload in *in vivo* systems .

Numerous studies have looked at the association between ascorbic acid intake and the risk of developing cardiovascular disease (CHD). A large prospective epidemiological study in Finnish men and women suggested that high intake of ascorbic acid was associated with a reduced risk of death from CHD in women and not in men [53]. Similarly, another study showed that high intake of ascorbic acid in American men and women appeared to benefit only women [54,55]. A third American cohort study suggested that cardiovascular mortality was reduced in both sexes by vitamin C [56]. In the UK, a study showed that the risk of stroke in those with highest intake of vitamin C was only half that of subjects with the lowest intake and no evidence suggestive of lower rate of CHD in those with high vitamin C intake [57]. However, a recent meta analysis on the role of ascorbic acid and antioxidant vitamins showed no evidence of significant benefit in prevention of CHD [58]. Thus, no conclusive evidence is available on the possible protective effect of ascorbic acid supplementation on cardiovascular disease.

### **Ascorbic acid and Cancer**

Nobel laureate Pauling and Cameron advocated use of high doses of ascorbic acid (> 10 g/day) to cure and prevent cold infections and in the treatment of cancer [34,59]. The benefits included were increased sense of well being/much improved quality of life, prolongation of survival times in terminal patients and

complete regression in some cases [60 -62]. However, clinical studies on cancer patients carried out at Mayo Clinic showed no significant differences between vitamin C and placebo groups in regard to survival time [63]. Cameron and Pauling [23] believed that ascorbic acid combats cancer by promoting collagen synthesis and thus prevents tumors from invading other tissues. However, researchers now believe that ascorbic acid prevents cancer by neutralizing free radicals before they can damage DNA and initiate tumor growth and may act as a pro-oxidant helping body's own free radicals to destroy tumors in their early stages [64 -66].

Extensive animal, clinical and epidemiological studies were carried out on the role of ascorbic acid in the prevention of different types of cancers. A mixture of ascorbic acid and cupric sulfate significantly inhibited human mammary tumor growth in mice, while administered orally [67]. Ascorbic acid decreased the incidence of kidney tumors by estradiol or diethylstilbestrol in hamsters due to decrease in the formation of genotoxic metabolites viz., diethylstilbestrol-4'-4''-quinone [68]. Ascorbic acid and its derivatives were shown to be cytotoxic and inhibited the growth of a number of malignant and non-malignant cell lines *in vitro* and *in vivo* [69,70 -72]. Ascorbic acid has been reported to be cytotoxic to some human tumor cells viz., neuroblastoma [73], osteosarcoma and retinoblastoma [74]. A number of ascorbic acid isomers/ derivatives were synthesized and tested on tumor cell lines. Roomi et al., 1998 [75] demonstrated that substitution at 2- or 6- and both at 2,6-positions in ascorbic acid have marked cytotoxicity on malignant cells. Ascorbate 6-palmitate and ascorbate 6-stearate, the fatty acid esters of ascorbic acid were found to be more potent inhibitors of growth of murine leukemia cells compared to ascorbate 2-phosphate, ascorbate 6-phosphate and ascorbate 6-sulfate respectively [75].

Among ascorbic acid derivatives, fatty acid esters of ascorbic acid viz., ascorbyl palmitate and ascorbyl stearate have attracted considerable interest as anticancer compounds in view of their lipophilic nature as they can easily cross

cell membranes and blood brain barrier [76]. Ascorbic acid and ascorbyl esters have been shown to inhibit the proliferation of mouse glioma and human brain tumor cells viz., glioma (U-373) and glioblastoma (T98G) cells and renal carcinoma cells [77-79]. Ascorbyl stearate was found to be more potent than sodium ascorbate in inhibiting proliferation of human glioblastoma cells [80]. Ascorbyl-6-O-palmitate and ascorbyl-2-O-phosphate-6-O-palmitate also showed anti-metastatic effect by inhibiting invasion of human fibrosarcoma HT-1080 cells through matrigel and pulmonary metastasis of mouse melanoma model systems [81].

Numerous reports are available in literature on cytotoxic and anti-carcinogenic effect of ascorbic acid and its derivatives in different tumor model systems. However, the molecular mechanisms underlying the anti-carcinogenic potential of ascorbic acid are not completely elucidated. Recently, Naidu et al [80] demonstrated that ascorbyl stearate inhibited cell proliferation by interfering with cell cycle, reversed the phenotype and induced apoptosis by modulation of insulin-like growth factor 1-receptor expression in human brain tumor glioblastoma (T98G) cells. They also studied the effect of ascorbyl stearate on cell proliferation, cell cycle, apoptosis and signal transduction in a panel of human ovarian and pancreatic cancer cells. Treatment with ascorbyl stearate resulted in concentration-dependent inhibition of cell proliferation and also clonogenicity of ovarian/pancreatic cancer cells [82,83]. The anti-proliferative effect was found to be due to the arrest of cells in S/G2-M phase of cell cycle, with increased fraction of apoptotic cells. The cell cycle perturbations were found to be associated with ascorbyl stearate induced reduction in the expression and phosphorylation of IGF-1 receptor, while the expression of EGFR and PDGFR remained unchanged. These changes were also associated with activated ERK1/2 but later reduction in AKT phosphorylation. Overexpression of IGF-1 receptor in OVCAR-3 cells had no protective effect, however ectopic expression of a constitutively active AKT2 did offer protection from the cytotoxic effects of ascorbyl stearate. In conclusion, ascorbyl stearate induced anti-proliferative and

apoptotic effects in ovarian cancer were found to be mediated through cell cycle arrest and modulation of the IGF-1R and PI3K/AKT2 survival pathways [83].

A plethora of epidemiological studies were carried out to find out the association of ascorbic acid with various types of cancers including breast, esophageal, lung, gastric, pancreatic, colorectal, prostate, cervical and ovarian cancer etc. The results were found to be inconclusive in most types of cancers except gastric cancer [84]. One of the most consistent epidemiological findings on vitamin C has been an association with high intake of ascorbic acid or vitamin C rich foods and reduced risk of stomach cancer. Considerable biochemical and physiological evidence suggests that ascorbic acid functions as a free radical scavenger and inhibit the formation of potentially carcinogenic N-nitroso compounds from nitrates, nitrite in stomach and thus offer protection against stomach cancer [85-87].

Low intake of ascorbic acid and other vitamins was associated with an increased risk of cervical cancer in two of three studies reported [88-91]. This relationship needs further study because the results suggest that other nutrients including vitamin E, carotenoids, retinoic acid either individually or in synergy with ascorbic acid may impart a protective effect against various cancers. Current evidences suggest that vitamin C alone may not be sufficient as an intervention in the treatment of most active cancers, as it appears to be preventive than curative. However, vitamin C supplementation has shown to improve the quality of life and extend longevity in cancer patients, hence it could be considered as an adjuvant in cancer therapy.

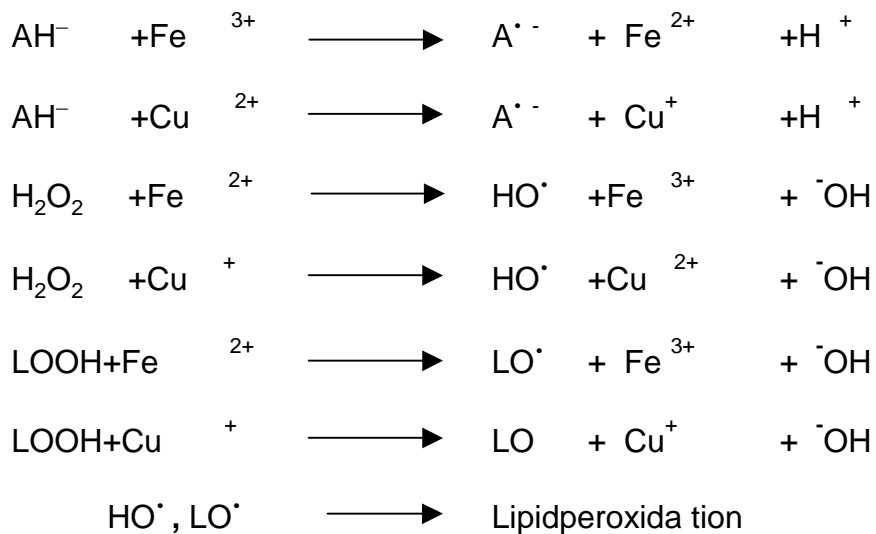
Dehydroascorbic acid, the oxidized form of ascorbic acid was shown to cross the blood brain barrier by means of facilitative transport and was suggested to offer neuroprotection against cerebral ischemia by augmenting antioxidant levels of brain [92].

## Controversies on health benefit of Ascorbic acid

### Does ascorbic acid act as an antioxidant or pro-oxidant?

Vitamin C is an important dietary antioxidant, it significantly decreases the adverse effect of reactive species such as reactive oxygen and nitrogen species that can cause oxidative damage to macromolecules such as lipids, DNA and proteins which are implicated in chronic diseases including cardiovascular disease, stroke, cancer, neurodegenerative diseases and cataractogenesis [93].

As shown in Table 2, ascorbic acid is a potent water-soluble antioxidant capable of scavenging/neutralizing an array of reactive oxygen species viz., hydroxyl, alkoxyl, peroxy, superoxide anion, hydroperoxyl radicals and reactive nitrogen radicals such as nitrogen dioxide, nitroxide, peroxy nitrite at very low concentrations [15]. In addition ascorbic acid can regenerate other antioxidants such as  $\alpha$ -tocopheroxyl, urate and  $\beta$ -carotene radical cation from their radical species [94]. Thus, ascorbic acid acts as co-antioxidant for  $\alpha$ -tocopherol by converting  $\alpha$ -tocopheroxyl radical to  $\alpha$ -tocopherol and helps to prevent the  $\alpha$ -tocopheroxyl radical mediated peroxidation reactions [95].



Adapted from Carr and Frei [15]

These radical species are highly reactive and can trigger lipid peroxidation reactions. Thus the question arises whether vitamin C acts as a pro-oxidant in *in vivo* conditions? The answer appears to be “no” although these reactions occur readily *in vitro*, its relevance in *in vivo* has been a matter of debate concerning ready availability of catalytically active free metal ions *in vivo* [94]. In biological systems, iron is not freely available, but it is bound to proteins like transferrin, hemoglobin and ferritin. Mobilization of iron from these biomolecules may be required before it can catalyze lipid peroxidation. Further, the concentration of free metal ions in *in vivo* is thought to be very low as iron and other metals are sequestered by various metal binding proteins [94]. Another factor that may affect pro-oxidant vs antioxidant property of ascorbic acid is its concentration. The *in vitro* data suggest that at low concentrations ascorbic acid acts as a pro-oxidant, but as an antioxidant at higher levels [96]. Moreover, a recent report demonstrated that large doses of exogenous iron (200mg) and ascorbic acid (75 mg) promote the release of iron from iron binding proteins and also enhance *in vitro* lipid peroxidation in serum of guinea pigs. This finding supports the hypothesis that high intake of iron along with ascorbic acid could increase *in vivo* lipid peroxidation of LDL and therefore could increase risk of atherosclerosis [52]. However, another study demonstrated that in iron-overloaded plasma, ascorbic acid acts as an antioxidant and prevents oxidative damage to lipids *in vivo* [97].

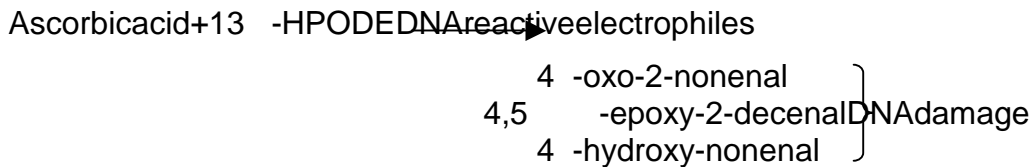
### **Is ascorbic acid harmful to cancer patients?**

Agus et al [98] have reported that the tumor cells contain large amounts of ascorbic acid, although the role of ascorbic acid in tumors is not yet known. They have established that vitamin C enters through the facilitative glucose transporters (GLUTs) in the form of dehydroascorbic acid, which is then reduced intracellularly and retained as ascorbic acid. It is speculated that high levels of ascorbic acid in cancer cells may interfere with chemotherapy or radiation therapy since these therapies induce cell death by oxidative mechanism. Thus, ascorbic acid supplementation might make cancer treatment less effective

because, ascorbic acid being a strong antioxidant may scavenge or neutralize the oxidative stress induced by chemotherapy in cancer patients. However, more studies are needed to understand the role of ascorbic acid in tumor cells and the speculative contraindication of ascorbic acid for cancer chemotherapy.

### Does ascorbic acid cause cancer?

Recently, it has been reported that lipid hydroperoxide can react with ascorbic acid to form products that could potentially damage DNA, suggesting that it may form genotoxic metabolites from lipid hydroperoxides implicating that ascorbic acid may enhance mutagenesis and risk of cancer. Lee et al [99], demonstrated that ascorbic acid induces decomposition of lipid hydroperoxide (13-(S)-hydroperoxy-(Z,E)-9,11-octadecadienoic acid; (13-HPODE) in presence of transition metals to DNA-reactive bifunctional electrophiles namely 4-oxo-2-nonenal, 4,5-epoxy-2-(E)-decenal and 4-hydroxy-2-nonenal. 4-oxo-2-nonenal being a genotoxin can react with DNA bases to form mutations [100] or apoptosis [101].



Thus, the above process can give rise to substantial amounts of DNA damage *in vivo*. However there are many questions, which need to be considered before we accept the hypothesis that ascorbic acid can cause cancer by producing genotoxic metabolites from lipids. The hydroperoxides formed through lipid peroxidation reaction are rapidly reduced to aldehydes by a number of enzymes. Further, ascorbic acid being a strong antioxidant effectively inhibits the formation of lipid peroxides as ascorbic acid forms the first line of antioxidant defense mechanism in human plasma. The formation of lipid hydroperoxides occur only after ascorbic acid has been exhausted. Hence, interaction of ascorbic acid and

hydroperoxide may not arise in human plasma. Recently, high intracellular vitamin C was reported to prevent oxidation-induced mutations in human cells [102]. Thus, the physiological relevance of these results is yet to be established in *in vivo* experiments.

## Conclusion

Ascorbic acid is one of the important and essential vitamins for human health. It is needed for many physiological functions in human biology. Fresh fruits, vegetables and also synthetic tablets supplement the ascorbic acid requirement of the body. However, stress, smoking, infections and burns deplete the ascorbic acid reserves in the body and demands higher doses of ascorbic acid supplementation. Based on available biochemical, clinical and epidemiological studies, the current RDA for ascorbic acid is suggested to be 100-120 mg/day to achieve cellular saturation and optimum risk reduction of heart diseases, stroke and cancer in healthy individuals. In view of its antioxidant property, ascorbic acid and its derivatives are widely used as preservatives in food industry. Many health benefits have been attributed to ascorbic acid namely antioxidant, anti-atherogenic and anti-carcinogenic activity. Lately some of these beneficial effects of ascorbic acid are contradicted. The relation between ascorbic acid and cancer is still a debatable as the molecular mechanism underlying anti-carcinogenic activity of ascorbic acid is not clearly elucidated. Regarding the pro-oxidant activity of vitamin C in presence of iron, there is compelling evidence for antioxidant protection of lipids by ascorbic acid both with and without iron co-supplementation in animals and humans. Current evidences also suggest that ascorbic acid protects against atherogenesis by inhibiting LDL oxidation. The data on vitamin C and DNA damage are conflicting and inconsistent. However, more mechanistic and human *in vivo* studies are warranted to establish the beneficial claims on ascorbic acid. Thus, though ascorbic acid was discovered in 17<sup>th</sup> century, the role of this important vitamin in human health and disease still remains a mystery in view of many beneficial claims and contradictions.

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Table 1. Ascorbic acid content in selected foods

Fruits	mg/100g edible portion
Banana	8-16
Apple	3-30
Mango	10-15
Pineapple	15-25
Cherry	15-30
Papaya	39
Orange	30-50
Grapefruit	30-70
Lemon	40-50
Strawberry	40-70
Currant black	150-200
Rosehips	250-800
<b>Vegetables</b>	
Onion	10-15
Tomato	10-20
Eggplant	15-20
Radish	25
Spinach	35-40
Cabbage	30-70
Cauliflower	50-70
Broccoli	80-90
Coriander	90
Brussels sprout	100-120
Pepper	150-200
Parsley	200-300

Adapted from Johnson et al [12]

Table 2. Reactive species scavenged by ascorbic acid

<b>Chemical species</b>	<b>Reaction rate (<math>M^{-1}s^{-1}</math>)</b>	
<b>Reactive oxygen species</b>		
Hydroxyl radical	$1.1 \times 10^{10}$	10
Alkoxy radical	$1.6 \times 10^9$	9
Peroxy radical	$1.2 \times 10^6$	6
Superoxide anion/hydroperoxy radical	$1.0 \times 10^5$	5
<b>Reactive nitrogen species</b>		
Dinitrogen trioxide/dinitrogen tetroxide	$1.2 \times 10^9$	9
Peroxynitrite/peroxynitrous acid	235	
<b>Antioxidant derived radicals</b>		
Alpha-tocopherol radical	$2 \times 10^5$	5
Urate radical	$1 \times 10^6$	6
Thiyl/sulphenyl radical	$6 \times 10^8$	8

Adapted from Carr and Frei [15]

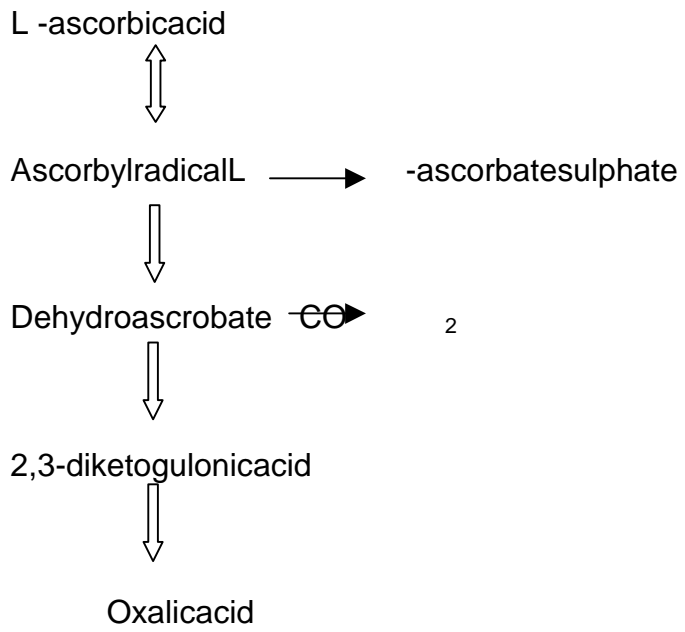


Fig1.CatabolismofAscorbicacid

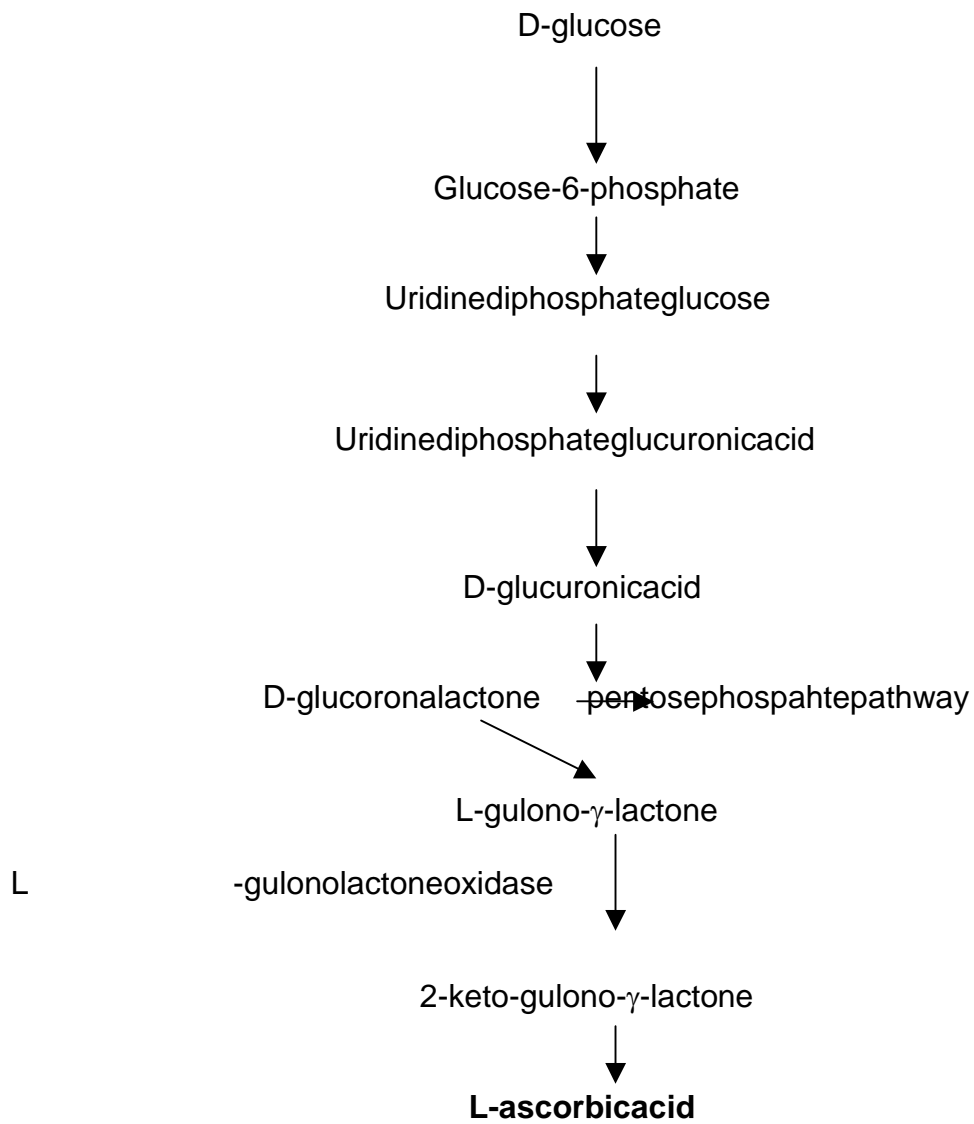


Fig2. Biosynthesis of L-Ascorbic acid in animals