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# Vitamin C: From nutrition to oxygen sensing and epigenetics

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A B S T R A C T

Vitamin C is unbeatable - at least when it comes to sales. Of all the vitamin preparations, those containing vitamin C sell best. This is surprising because vitamin C deficiency is extremely rare. Nevertheless, there is still controversy about whether the additional intake of vitamin C supplements is essential for our health. In this context, the possible additional benefit is in most cases merely reduced to the known effect as an antioxidant. However, new findings in recent years on the mechanisms of oxygen-sensing and epigenetic control underpin the multifaceted role of vitamin C in a biological context and have therefore renewed interest in it. In the present article, therefore, known facts are linked to these new key data. In addition, available clinical data on vitamin C use of cancer therapy are summarized.

## 1. Introduction

The terminus vitamin is commonly used to describe organic molecules, or their derivatives, that are essential for the proper life and functioning of an organism. Such essential molecules cannot be synthesized by the organism itself, or not in sufficient quantity, and need therefore to be ingested via the diet. In humans, a general distinction is made between the group of fat-soluble and water-soluble vitamins.

With the term vitamin C we refer commonly to a water-soluble organic compound known as ascorbic acid (IUPAC name; (5 R)-5-[(1S)-1,2-Dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one) or its oxidized form dehydro ascorbic acid (DHA). When the term vitamin C or ascorbic acid is used, it usually refers to only one of four stereoisomers, namely L-(+)-ascorbic acid, since only this isomer possesses the biological activity typical of vitamin C.

In humans both ascorbic acid and its oxidized form DHA are cofactors for many biochemical reactions which it plays the role of an electron donor. Due to its "a-scorbic" or anti-scurvy activity, it is well known to be necessary for formation of connective tissue (collagen), bones, skin, gums, and teeth where vitamin C is an important cofactor for hydroxylation of prolyl and lysyl residues in collagen. Vitamin C also contributes to the normal functioning of cholesterol metabolism, the synthesis of carnitine, and hydroxylation of steroids [1]. In addition, it is required for the synthesis of serotonin, and conversion of dopamine to norepinephrine, making it contributing to the reduction of fatigue and exhaustion, and to normal mental function. Further, vitamin C improves ingestion of iron especially from plant foods and appears to contribute to improve the immune system after heavy exercise.

Through its electron-transferring action, vitamin C first became famous as an antioxidant. In the meantime, it is known that very high pharmacological doses of vitamin C also seem to have a prooxidative effect. As an antioxidant vitamin C can scavenge free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). Accordingly, vitamin C is considered one of the most important antioxidants in plants and animals. In addition to direct ROS neutralization, ascorbic acid is crucial for regeneration of other antioxidant food substances, e.g., vitamin E. This makes vitamin C an important redox node, particularly in conjunction with glutathione [2]. In line with its antioxidant capabilities, vitamin C has also been demonstrated to influence important intracellular signal transduction pathways. Thereby, vitamin C appears to be an important cofactor for enzymes that regulate the activity of transcription factors such as hypoxia-inducible factor-1α (HIF-1α) or nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) that are activated in the context of physiological or pathological environmental conditions such as oxygen deprivation or inflammation [3]. Recent evidence also indicates that vitamin C can influence the epigenetic program of cells by participating in enzyme reactions that modify histone and DNA methylation patterns in response to external influences such as stress, disease, or diet.

Therefore, the aim of this review is to shed light on the role of vitamin C in the context of human nutrition and its molecular aspects up to gene regulation and epigenetics.

## 2. Discovery

In the 16th to 19th centuries, scurvy (latin, scorbutus) was a dangerous disease that occurred primarily in sailors who were at sea for months at a time and whose diet did not contain fresh fruits and vegetables. In 1747, James Lind conducted an experiment to treat scurvy with lemon and orange, which is considered the first clinical trial in

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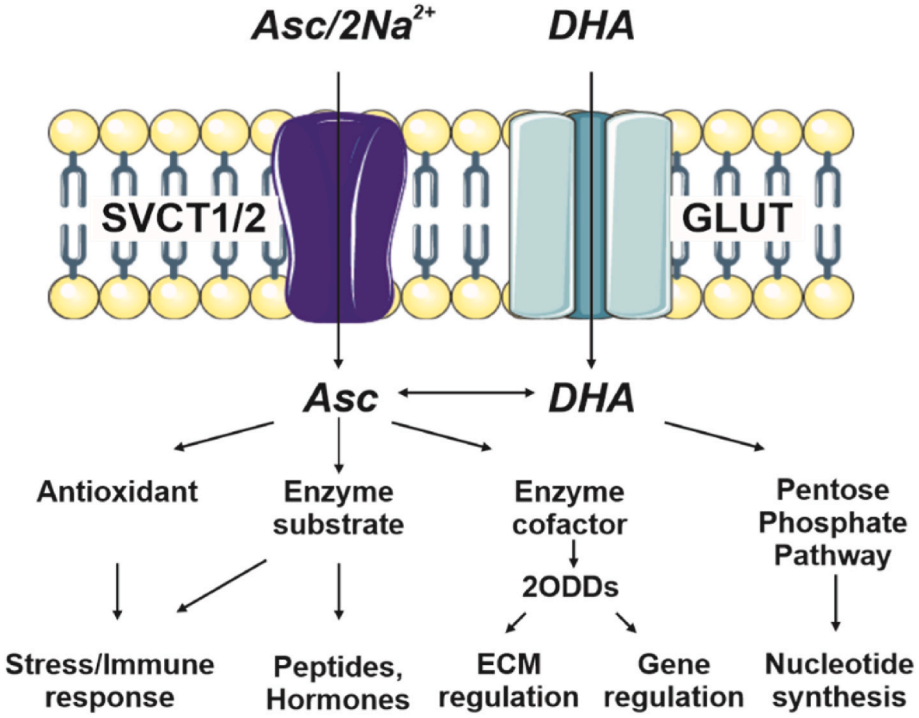


**Fig. 1.** Cellular uptake and use of ascorbate. Ascorbate (Asc) is taken up by human cells with the help of the sodium-dependent vitamin C transporters SVCT1 and SVCT2. Dehydroascorbate (DHA) can be transported by different glucose (GLUT) transporters depending on cell type. Within cells ascorbate can serve as antioxidant, as enzyme substrate in reactions generating peptides and hormones such as dopamine. As cosubstrate in 2-oxoglutarate-dependent dioxygenase (2ODDs) reactions it takes part in ECM regulation via e.g., collagen synthesis as well as gene regulation by modulating HIF prolyl hydroxylases and histone demethylases. DHA catabolism can, via the pentose phosphate pathway, contribute to nucleotide synthesis by generating xylulose 5-phosphate. (See text for details).

history [4]. Later, citrus juice was used by British sailors to avoid scurvy.

In 1907, Holt and Frølich made a major advance by successfully producing scurvy in guinea pigs [5]. As a result, the guinea pig became a common animal model in scurvy research. Using the guinea pig model, it was found that the cause of scurvy was a deficiency of a "vitamin" [6]. The corresponding causal vitaminic agent was first isolated in 1927 and named "hexuronic acid" by Albert Szent-Györgyi [7,8]. The actual structure of "hexuronic acid" was then presented by Norman Haworth in 1933. Alluding to the antiscorbutic properties of the compound, Haworth and Szent-Györgyi proposed renaming it into "a-scorbic acid" and later more specifically to L-ascorbic acid. For their research on vitamin C, Haworth and Szent-Györgyi received the Nobel Prize in Chemistry and in Medicine or Physiology in 1937, respectively.

## 3. Synthesis

The chemical synthesis of ascorbic acid from glucose was successful for the first time in 1933. In this process, D(+)-glucose is first reduced to sorbitol and then oxidized in bacteria, *e.g.*, *Acetobacter suboxidans*, to L (−)-sorbose. The latter is further oxidized to diacetone-L-sorbose with the addition of acetone. After the subsequent cleavage of the acetone, 2-keto-L-gulonic acid is formed from which ascorbic acid is generated after cleavage with water. With some minor modifications the same method has been used to produce vitamin C on an industrial scale for several decades [9]. Recently, the production of vitamin C by one-step fermentation with the help of genetically modified organisms has become attractive due to its simplification [10,11].

The biosynthetic pathways contributing to ascorbate biosynthesis in plants, animals, photosynthetic protists and fungi have been extensively reviewed elsewhere [12–15]. As plants are the major vitamin C source for humans its synthesis is briefly reflected below.

Plant vitamin C biosynthesis occurs enzymatically by using D-mannose and L-galactose also called the Smirnoff-Wheeler pathway [14,16,17]. The pathway starts by converting glucose into D-glucose-6--phosphate into GDP-D-mannose through the reaction of hexose phosphate isomerase, phospho-mannose isomerase, phospho-mannose mutase, and GDP-D-mannose phosphorylase. GDP-D-mannose is transformed into GDP-L-galactose by GDP-mannose-3′,5′-epimerase. Thereafter, the sequential action of GDP-L-galactose phosphorylase, L-galactose-1-phosphate phosphatase, L-galactose dehydrogenase, and finally L-galactono-1,4-lactone dehydrogenase led to formation of ascorbic acid. Interestingly, the GDP-mannose 3′,5′-epimerase also catalyzes a reaction converting GDP-D-mannose into GDP-L-gulose, making a branch of L-ascorbic acid synthesis in plants by using L-gulose as intermediate [18]. Ascorbic acid can also be synthesized from D-galacturonic acid in rotten strawberry with D-galacturonate reductase as the key step [19].

In plants and other photosynthesizing organisms, ascorbate's major role is to protect photosynthetic cells against ROS derived from the chloroplasts [20].

While primates (including humans), guinea pigs and teleost fishes, as well as some families in the orders of fruit bats and passerine birds, are not capable of biosynthesizing ascorbic acid, those vertebrates that produce ascorbic acid, do it mostly in liver and kidney. Thereby the synthesis of ascorbic acid starts with the oxidation of UDP-D-glucose to UDP-D-glucuronic acid by the enzyme UDP-glucose dehydrogenase. The oxidizing agent in this process is $NAD^+$. After hydrolytic cleavage of UDP, D-glucuronic acid is formed, which is converted to L-gulonic acid by regioselective reduction by glucuronate reductase and $NADPH+H^+$. Lactonization, i.e., formation of L-gulano-1,4-lacton is achieved by gluconolactonase. Thereafter, L-gulonolactone oxidase in the presence of oxygen and FAD as a cofactor performs a selective oxidation and forms $H_2O_2$ and 2-keto-L-gulonlacton that spontaneously isomerizes to L-ascorbic acid [21].

In all animals whose lives depend on exogenous vitamin C supplementation, expression of the *GULO* gene encoding L-gulonolactone oxidase is lost due to several loss-of-function mutations. To date, there is no satisfactory evolutionary explanation for the apparently random loss of vitamin C synthesizing ability. However, several hypotheses exist which try to explain the evolutionary lack of ascorbate synthesis with benefits to these species [22–24]. Although no clear evidence has been presented, one sensible explanation could be that lack of GULO activity reduces formation of $H_2O_2$ and other subsequently formed ROS that could harm the organism. At the same time, the *GULO* lack would increase the pool of available UDP-D-glucuronic acid that serves as a precursor in proteoglycan formation and glucuronidation reactions that

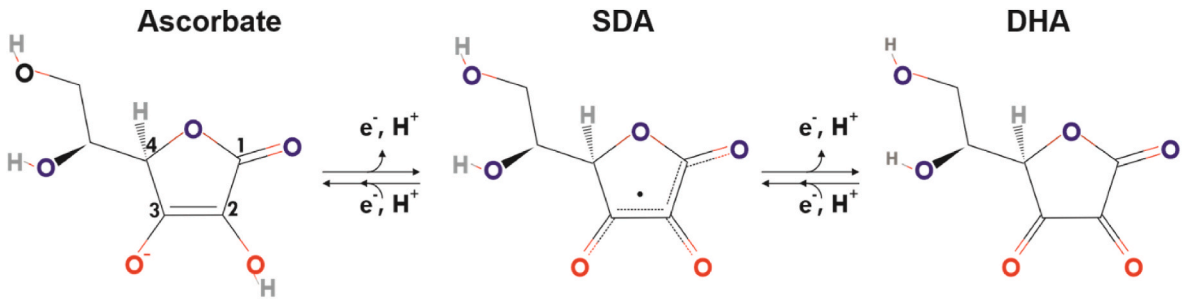


**Fig. 2.** The three vitamin C redox states. At physiological pH vitamin C exists as ascorbate anion in its fully reduced form. Donation of an electron yields SDA• (semidehydroascorbate) which represents the monooxidized radical form; donation of another electron generates DHA (dehydroascorbate), the fully oxidized form).

eliminate lipophilic xeno- and endobiotics [12]. Another loss of vitamin C synthesis could have been caused by genetic drift, in which mutations in the *GULO* gene were passed on because they were overlooked during natural selection. Interestingly, the animals that lack the ability to synthesize vitamin C are phylogenetically unrelated, suggesting that no changes in human lifestyle (e.g., long voyages at sea) made it a vitamin, but rather that species-independent mutations occurred that all resulted in the same phenotype. This was apparently favoured by the fact that the diet of the species in question was rich in vitamin C for a long time, which compensated for the enzyme loss [25,26].

## 4. Absorption, transport, metabolism

Vitamin C is ingested in jejunum and ileum. Human cells seem to store and to reabsorb vitamin C to a certain extent, so that the body's own reserves are sufficient for about 2–6 weeks. In the blood, about 24% of vitamin C is bound to protein.

The uptake of ascorbate into the interior of human cells occurs along with two sodium ions each by the sodium-dependent vitamin C transporters SVCT1 and SVCT2 [27–29], respectively (Fig. 1). Both SVCT1 and SVCT2 seem to transport only ascorbate [30,31], and differ in their affinity and capacity towards ascorbate. Thereby, SVCT1 seems to represent a high-capacity system with low affinity whereas SVCT2 is a low-capacity transporter with high affinity [30,31].

The main excretion of ascorbate occurs via the kidney through the urine. Again, SVCT1 appears to be crucial in excretion and reabsorption as knockout of the SVCT1 encoding Slc23a1 gene in mice causes loss of about 70% ascorbate in tissues via urinary elimination [32]. Interestingly, mice lacking SVCT2 died almost immediately after birth due to brain hemorrhage [33]] suggesting SCVT2 being important for brain ascorbate supply and vascular development. However, genome wide association studies with single nucleotide polymorphisms in the human *SLC23A1* and *SLC23A2* genes did neither reveal clinical significant disease associations nor alterations in the vitamin C status [34].

In contrast to ascorbate, the oxidized derivative DHA appears to be transported by glucose transporters GLUT2 and GLUT8 from intestine into blood [35], and by GLUT-1, GLUT-3, and GLUT-4 [28,36] from blood to tissues as intestinal cells do not express these transporters. During this process, excess glucose can competitively prevent the uptake of DHA. Within cells, ascorbate is quickly regenerated from DHA with the help of glutathione and NADPH [12]. This process both maintains a substantial DHA gradient for GLUTs and generates a compound that is no longer a GLUT substrate [28]. Although many details about the involvement of GLUTs in DHA transport are lacking, their role may contribute to a "bystander effect" in which extracellular DHA can be taken up instead of ascorbate when the latter has been oxidized, e.g., by superoxide released from neighboring cells in an oxidative burst [37]. In addition, at physiological conditions DHA undergoes rapidly hydrolysis into 2,3-diketo-gulono acid that has a half-life of 6–7 min and gives rise to the formation of L-xylonate and L-lyxonate or L-erythrulose and oxalate. While L-xylonate and L-lyxonate can enter the pentose phosphate pathway, L-erythrulose is supposed to participate in glycation of lens proteins [38,39] whereas oxalate is eliminated via the urine. Interestingly, when humans where injected with labeled ascorbate, oxalate appeared to be the major urinary metabolite (~44%), followed by 2, 3–diketo-gulono acid (~20%), ascorbate (~20%), and DHA (<2%) [40] which led to the suggestion that oxalate may have potential to crystallize as calcium oxalate in kidneys and urine of susceptible people [41].

## 5. Nutrition facts

The recommended intake of vitamin C depends on age and, from puberty onwards, also on gender. For infants and children under 4 years of age, the recommended intake is 20 mg per day, for 13- to 15-year-olds 85 mg per day. In male 15- to 25-year-olds, the recommended daily intake is 105 mg, and in females 90 mg. In later adult life (25 to < 51 years) the recommended daily vitamin C intake is 95 mg (women) and 110 mg (men). From the fourth month onwards, pregnant women should have a daily intake of 105 mg, and breastfeeding women a daily intake of 125 mg of vitamin C. Due to increased cigarette smoking caused formation of free radicals, female smokers are recommended to take 135 mg per day and male smokers 155 mg per day [42].

Foods containing vitamin C are mainly fruits and vegetables and their juices or smoothies. Good sources of vitamin C are, for example, sweet peppers, black currants, sea buckthorn, parsley, kale, broccoli, fennel, citrus fruits, rose hips or garden cress [43].

In the food industry, ascorbic acid is used as antioxidant and color stabilizer in canned fruit and vegetables, frozen or dried potato products, fruit juices and nectars, jams, meat, sausage, and fish products, as well as bread and baking mixes. For example, apple juice should not turn brown, and sausage should remain beautifully red. Furthermore, ascorbic acid inhibits the formation of nitrosamines in cured products and improves the dough properties of flours [44].

The vitamin C content of foods varies depending on several factors. For example, the time of harvest, transport, type and duration of storage, and preparation in the kitchen are decisive factors. Improper storage (e.g., air) as well as processing in the kitchen (e.g., leaching by water) can lead to high vitamin C losses. Likewise, enzymatic processes in fruits and vegetables can reduce vitamin C content. Blanching (brief boiling in water) counteracts enzymatic vitamin C loss [44].

In industrialized countries, vitamin C deficiency practically does no longer occur. Classic clinical vitamin C deficiency states are Moeller-Barlow disease in infants and scurvy in adults (in the past described as "sailor's disease"). In these cases, bone formation and growth are disturbed in the infant and child. In later stages of life, symptoms include tooth loss, joint pain, infections, poor wound healing, and bleeding tendencies in skin, muscles, internal organs, and mucous membranes. These disorders occur in adults only in the event of a permanent lack of vitamin C intake. Already daily provision of 10 mg vitamin C prevents scurvy [42,44,45].

No hypervitaminoses are known for vitamin C. Extremely high doses (from 3 to 4 g per day) can lead to gastrointestinal complaints (e.g.,

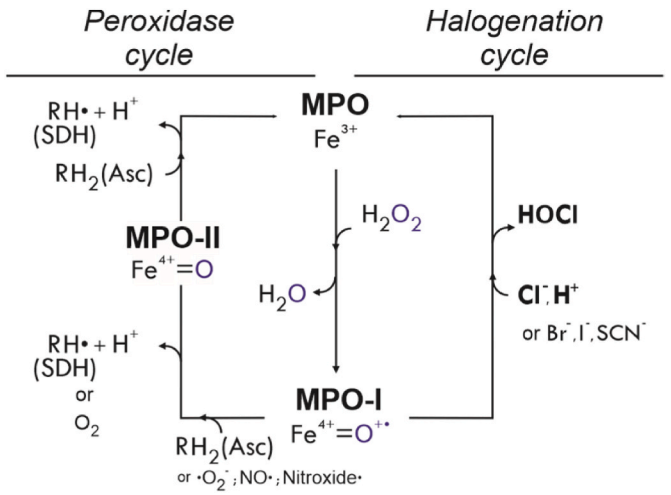


**Fig. 3.** Scheme of myeloperoxidase (MPO) reaction cycles. In the halogenation cycle NADPH oxidase derived $H_2O_2$ oxidizes MPO to generate MPO-I. MPO-I in turn will be reduced by the electrons from the halides that are at the same time converted into hypohalous acids, e.g., hypochlorous acid. In addition to halides, the MPO-I compound also oxidizes nitrite ($NO_2^-$), nitric oxide (NO), thiocyanate (SCN-). In the peroxidase cycle MPO-I undergoes two subsequent one-electron reductions in order to regenerate the native ferric enzyme; vitamin C, *i.e.*, ascorbate among others can act as electron donor. For further details see text.

diarrhea). There are some types of people who are at increased risk for harmful vitamin C side effects. These include persons with kidney damage, persons with a predisposition to urinary or kidney stones, G6PDH deficiency, or with disorders in the utilization of dietary iron (hemochromatosis, hemosiderosis, thalassaemia major). However, even for them an intake of up to 1 g per day can be tolerated without harmful side effects [46–48].

## 6. Ascorbate and reactive oxygen species (ROS)

With two pKa's, at 4.25 and 11.6 [49], ascorbic acid is strongly acidic and therefore exists under physiological pH conditions as ascorbate anion. The protons are released at the two OH groups of the ring system, with the hydroxyl group at the third carbon atom of the ring releasing its proton more easily than the neighboring hydroxyl group at the second carbon atom.

Ascorbate is able to neutralize/reduce free radicals which frequently occurs in a process where ascorbate donates first an electron to reduce the radical and becomes itself oxidized to an ascorbyl radical (mono- or semidehydroascorbate; SDA); *e.g.*, 2 L-ascorbate + $H_2O_2$ + 2 $H^+$ → 2 SDA• + 2$H_2O$, or 2 L-ascorbate + an oxidized electron carrier + 2 $H^+$ → 2 SDA• + a reduced two electron carrier.

Then, when SDA is donating an electron again, DHA is formed [50] (Fig. 2). Further, it is also possible for two ascorbyl radical molecules to disproportionate into ascorbic acid and DHA. Overall, vitamin C is supposed to contribute to up to 30% of the "total antioxidant power" in blood [51] (see Fig. 3).

Despite its ability to reduce radicals, ascorbate can be also a pro-oxidant. This occurs usually in a very slow autooxidative process of ascorbate at neutral pH, or in a rapid process catalyzed by metals [52]. In either case, oxidation of ascorbate forms $H_2O_2$, which can affect cells by altering their intracellular redox homeostasis [49,52]. The respective effect of acting as a reducing or oxidizing factor is dependent on the vitamin C concentration in plasma.

Physiological concentrations of ascorbic acid have mainly antioxidant effects, while very high concentrations are associated with prooxidant actions [49].

**Table 1**
Human enzymes using ascorbate derivatives as substrate.

| EC number | Enzyme name | Reaction |
|---|---|---|
| 1.6.5.4 | monodehydroascorbate reductase (NADH) | NADH + $H^+$ + 2 SDA• → NAD+ + 2 ascorbate |
| 1.8.5.1 | glutathione dehydrogenase (ascorbate) | 2 glutathione + dehydroascorbate → glutathione disulfide + ascorbate |
| 1.11.2.2 | Myeloperoxidase | Halogenation: Cl- + $H_2O_2$ + H+ → HOCl + $H_2O$<br>Peroxidase: $H_2O_2$ + 2$AH_2$ (ascorbate) → 2SDA• + 2$H_2O$ |
| 1.14.17.1 | dopamine beta-monooxygenase | Dopamine + 2 ascorbate + $O_2$ → noradrenaline + 2 SDA• + $H_2O$ |
| 1.14.17.3 | peptidylglycine monooxygenase | [peptide]-glycine + 2 ascorbate + $O_2$ → [peptide]-(2S)-2-hydroxyglycine + 2 SDA• + $H_2O$ |
| 7.2.1.3 | ascorbate ferrireductase (transmembrane) | Ascorbate [side 1] + Fe(III) [side 2] → SDA• [side 1] + Fe(II) [side 2] |

## 7. Enzymes using ascorbate as substrate

Although ascorbate's role today is more associated with its proper role as an antioxidant, it participates at the same time with this role in enzyme reactions. In this context, reactions in which ascorbate, semi-dehydroascorbate, or DHA itself act as substrates (Table 1) can be distinguished from those in which ascorbate is an essential cofactor.

The mechanisms and enzymes used to reconstitute ascorbate from semidehydroascorbate, or DHA appear to depend on the cell type, and the cellular compartment. Enzymes such as the outer mitochondrial membrane located NADH-cytochrome b5 reductase (EC 1.6.2.2) or cytoplasmic thioredoxin reductase (EC 1.8.1.9.) are able to convert semidehydroascorbate back to ascorbate in one-electron donating steps [53,54]. Likewise, DHA can be reduced back to ascorbate in two-electron donating steps by various enzymes such as glutaredoxin-1 (EC 1.8.5.1), protein disulfide isomerase (EC 5.3.4.1) [55,56], omega class glutathione transferase (EC 2.5.1.18) [57,58], 3α-hydroxysteroid dehydrogenase (EC 1.1.1.213) [57], or NADPH thioredoxin reductase (EC 1.8.1.9) [59]. Other enzymes where ascorbate or its derivatives are used as a substrate are monodehydroascorbate reductase (NADH) (EC 1.6.5.4) [60], glutathione dehydrogenase (ascorbate) (EC 1.8.5.1), L-ascorbate oxidase (EC 1.10.3.3), and transmembrane ascorbate ferrireductase (EC 7.2.1.3) as well as spontaneous reaction with GSH [61–63].

In addition to the above-mentioned reactions, which are mainly recycling ascorbate, ascorbate also participates as substrate in several other reactions that are important for human life (Table 1).

For example, ascorbate functions as a physiological reductant for the Cu2+-dependent dopamine β-hydroxylase- (1.14.17.1 dopamine beta-monooxygenase) enzyme. The enzyme, that is mainly found in the chromaffin granules of the adrenal medulla, generates norepinephrine from dopamine. In the reaction, one oxygen atom from molecular $O_2$ is added to the beta-carbon in dopamine, and the second oxygen atom goes into water. The required two electrons are provided by two ascorbates that become oxidized to SDA• [64,65].

Likewise, the peptidyl glycine α-amidating monooxygenase (EC1.14.17.3 peptidylglycine monooxygenase) is also a Cu2+-dependent monooxygenase that resides in the pituitary glands. The enzyme catalyzes the amidation of carboxyl-terminal glycine residues in various peptides of which the best substrates are peptidylglycines with a neutral amino acid residue at the penultimate position. To execute its function, the enzyme requires high concentrations of ascorbate. The product of the reaction undergoes immediate peptidylamidoglycolate lyase (EC 4.3.2.5) catalyzed dismutation into the corresponding desglycine peptide amide and glyoxylate. In mammals, the two activities of EC1.14.17.3 and EC 4.3.2.5 reside in a bifunctional protein that also participates in alpha-melanotropin synthesis [66,67].

Another enzyme of importance is the myeloperoxidase (MPO; EC 1.11.2.2) which belongs to the family of mammalian heme peroxidases. It is a calcium containing complex that exists as heterotetramer covalently bound to heme; the tetramer is composed of two light chains and two heavy chains.

MPO is expressed in neutrophil granulocytes, monocytes, various tissue macrophages such as Kupffer cells and cells of microglia, and in Alzheimer's patients also in neurons [68]. In neutrophil granulocytes and monocytes, MPO is found in azurophilic granules where it can account for about five percent of the total cell protein. After phagocyte activation, i.e., the oxidative burst, MPO is secreted into phagolysosomal compartments and then into the extracellular space where the absorption properties of MPO contribute significantly to the greenish-yellow color of the pus. Hence, MPO activity is linked with various inflammatory conditions such as oxLDL formation, cardiovascular disease and atherosclerosis as well as thyroiditis [69–77].

MPO is unusual as it oxidizes several substrates by various mechanisms [78] that involve formation of at least two, sometimes three intermediary compounds (MPO-I, MPO-II, and MPO-III). It catalyzes generation of hypochlorous-, hypobromous, or hypoiodous acid from chloride, bromide or iodine in the presence of $H_2O_2$ [79]. The $H_2O_2$ for the MPO reaction is generated by dismutation of superoxide ($O_2$•-) that is formed during the oxidative burst by the concurrently activated NADPH-oxidase (NOX). In the so-called halogenation reaction, MPO becomes first "oxidized/activated" by $H_2O_2$ and forms MPO-I, i.e., the initial Fe3+ in the heme is converted into a oxoiron(IV) species and a porphyrin radical-cation. A following two-electron reduction of MPO-I by halides will result in the production of the afore mentioned hypohalous acids and reconstitution of the resting ferric state in MPO. In addition to halides, the MPO-I compound also oxidizes nitrite ($NO_2^-$), nitric oxide (NO), thiocyanate (SCN-), and tyrosine [75,76,80]. Further, MPO-I can act as a classical peroxidase [78] where a first one-electron reaction reduces MPO-I to MPO-II, which is reduced in a second reaction again donating one-electron. As a result, the native ferric enzyme is regenerated. Each step in the reaction is accompanied by the release of a substrate radical. In addition to ascorbate, substrates for peroxidation may include serotonin, tyrosine, and exogenous aromatic amines and phenols [80–83]. Moreover, superoxide formed during the oxidative burst may give rise to generation of MPO-III; a complex of ferrous-dioxygen in resonance with ferric superoxide [68,84].

Overall, it catalyzes the reaction: $Cl^- + H_2O_2 + H^+ \rightarrow HOCl + H_2O$ in the halogenation cycle and as a peroxidase it can catalyze: $H_2O_2 + 2AH_2$ (ascorbate) $\rightarrow 2SDA\bullet + 2H_2O$

## 8. 2-Oxoglutarate-dependent dioxygenases (2ODDs)

The initial understanding of ascorbate function in humans stems from the various symptoms of scurvy associated with a deficiency of collagen. Collagens constitute the major components of the extracellular matrix (ECM). From the 28 currently known collagens, type I collagen appears to be the major structural protein of the interstitial matrix, whereas type IV predominates in basal membranes [85].

Although several posttranslational modifications contribute to the assembly of collagen molecules, its proper formation is strictly dependent on the collagen prolyl-4-, and prolyl-3-hydroxylases as well as lysyl hydroxylases 1 and -2 which are located in the lumen of the endoplasmic reticulum [86–88]. The resulting hydroxyprolines function to form hydrogen bonds between adjacent collagen polypeptide chains which strengthens the triple helical collagen molecule, while hydroxylysines are the prerequisite for covalent cross-links between collagen molecules. Subsequent studies then revealed that the hydroxylases belong to the group of $Fe^{2+}$-2-oxoglutarate-dependent dioxygenases (2ODDs) [89,90]. Importance of this enzyme family is underlined by the fact that it is

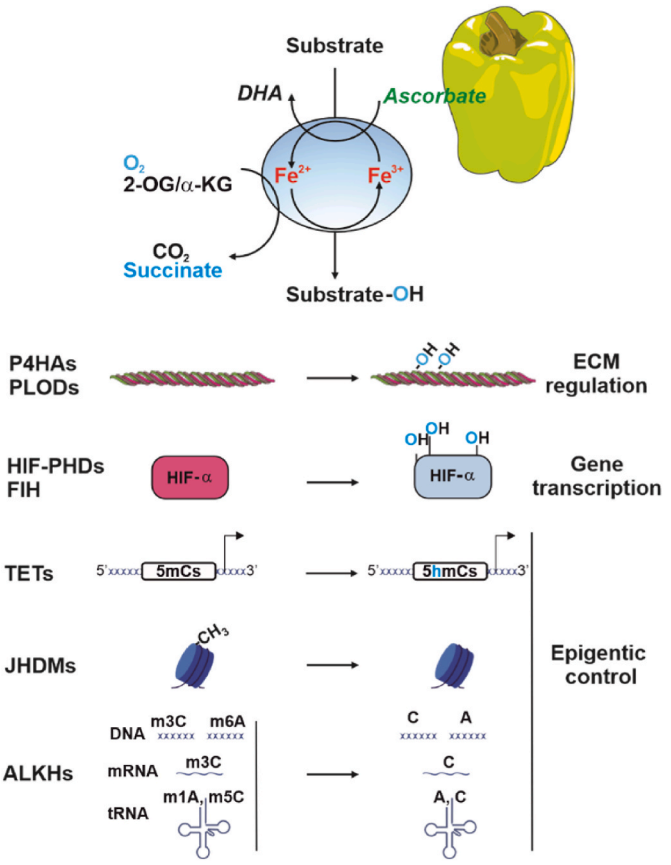


**Fig. 4.** Role of ascorbate in 2-oxoglutarate-dependent dioxygenase (2ODDs) reactions along with functional consequences mediated by key 2ODD family members. The various 2ODDs have multiple different substrates but all require divalent non-heme iron ($Fe^{2+}$) and use oxygen ($O_2$) and 2-oxoglutarate (2-OG) also known as α-ketoglutarate (α-KG) as co-substrates as well as ascorbate as cofactor. In the overall reaction, these enzymes incorporate one oxygen atom from $O_2$ into the product while the other helps to form succinate that is released together with $CO_2$. Note, the hydroxymethyl group generated during oxidative demethylation is spontaneously converted into formaldehyde to liberate the methyl carbon group (not shown). P4HAs, Procollagen prolyl 4-hydroxylase alpha subunits; PLODs (Procollagen-Lysine, 2-Oxoglutarate 5-Dioxygenases); HIF-PHDs, HIF-prolyl hydroxylases; FIH, Factor inhibiting HIF; TETs, ten-eleven translocation proteins; JHDMs, Jumonji-C domain-containing histone demethylases; ALKHs, mammalian AlkB homologs of DNA oxidative demethylases. For further details see text.

closely linked to intermediary metabolism as 2-oxoglutarate, that is equivalent to α-ketoglutarate, is a key intermediate in the tricarboxylic acid (TCA)/citric acid/Krebs cycle, where it is formed from isocitrate and gives rise to generation of succinyl CoA.

In addition to collagen, the members of this dioxygenase family have manifold substrates including, but not limited to, *e.g.,* DNA, RNA, and fatty acids. In humans ~70 family members having different subcellular locations are known [91–93]. Apart from the different substrates, they all require divalent non-heme iron ($Fe^{2+}$) and use oxygen ($O_2$) and 2-oxoglutarate as co-substrates as well as ascorbate as cofactor. In the overall reaction, these enzymes incorporate one oxygen atom from $O_2$ into the product while the other helps to form succinate that is released together with $CO_2$ (Fig. 4).

Substrate + 2-oxoglutarate + $O_2$ → Hydroxyl-substrate + succinate + $CO_2$

Although the general chemical mechanism of 2ODD reactions in terms of hydroxylation is quite well understood, several mechanistic details with respect to complex kinetics and dynamics in the entire world of 2ODDs are still to be discovered (please see several excellent recent

reviews [91,92,94–97].

The consensus catalytic mechanism of 2ODDs appears to occur in an ordered sequence. Although the processes by which divalent iron is incorporated to the active site of the 2ODDs are not well understood, $Fe^{2+}$ coordinates 2-oxoglutarate binding in the first reaction step. In turn, 2-oxoglutarate binding results in release of two water molecules as the iron coordinates binding of three water molecules in addition to binding the enzyme; commonly to a motif consisting of two histidines and one carboxylate (E/D) amino acid. Next, binding of the primary substrate to a site discrete from the metallocentre weakens binding of the third $Fe^{2+}$-ligated water, and its release enables binding of $O_2$. Subsequently, oxidative decarboxylation of 2-oxoglutarate forms succinate, $CO_2$, and leaves a ferryl ($Fe^{4+}$=O) species in the active center of the enzyme that reacts with the substrate's C–H bond to hydroxylate it. Interestingly, in the absence of substrate binding, the enzyme may undergo an uncoupled reaction in which the iron would be locked in the high oxidation state [92,98].

Ascorbate's function in the 2ODD reactions is to reduce iron back to maintain its divalent state, thereby maintaining the full activity of this class of enzymes [91,92,94,95]. Although ascorbate is supposed to be the main reductant in these enzyme reactions that usually cannot be replaced [99,100] there are some members with which glutathione was able to maintain activity [101]. However, the mechanistic details about ascorbate's exact role, in relation to $Fe^{4+}$=O intermediate generation and reduction as well as $O_2$ activation, oxidative rearrangements, are so far unknown.

The recent discoveries that 2ODD members participate in vital processes such as oxygen-dependent transcriptional regulation and epigenetics has also led to a renaissance in the interest of ascorbate.

### 8.1. 2ODDs that control oxygen-dependent transcription

Oxygen deficiencies trigger adaptation responses that enable long-term adaptation through an altered gene expression profile. This is regulated by hypoxia-inducible transcription factors (HIFs). HIFs are αβ-heterodimeric transcription factors that regulate more than 300 genes whose products balance oxygen supply and demand. There are three α-subunits, HIF-1α, HIF-2α, HIF3α, that are susceptible to proteasomal degradation under normoxia, while the β-subunit (HIF-1β/ARNT) is stable [102–105].

The normoxic degradation of the HIF α-subunits can be triggered by three HIF-proline 4 hydroxylase isozymes (known as HIF–P4H, PHDs, or EglNs; EC 1.14.11.29) that hydroxylate one or two proline residues within HIFαs which earmark them for ubiquitinylation by the von Hippel-Lindau (pVHL) tumor suppressor protein complex and subsequent proteasomal degradation.

In addition to the HIF-proline 4 hydroxylases, the asparagine hydroxylase factor inhibiting HIF (FIH; EC 1.14.11.30) hydroxylates an asparagine residue in HIF-1α and HIF-2α. As a result, under normoxic conditions binding of the coactivators CBP/p300 is inhibited along with HIF target gene transcription [106–108].

As the catalytic activity of HIF-proline 4 hydroxylases and the asparagine hydroxylase is oxygen-dependent, they are inactivated by hypoxia, leading to HIFα stabilization cofactor recruitment and target gene activation. This includes, *e.g.*, enhanced erythropoiesis and angiogenesis, modulation of inflammation, and reprogramming of metabolism [104,109–111].

Despite basic similarities, there are also specific differences between HIF–P4H and HIF isoforms in cells or organs. For example, the HIF-proline 4 hydroxylase 2 (HIF–P4H-2/PHD2/EglN1) is the major contributor for HIFα stability. Further, there is an N-terminal and C-terminal proline hydroxylation site in the HIF proteins (*e.g.*, P402 and P564 in HIF-1α), of which the C-terminal can be hydroxylated equally by all HIF-proline 4 hydroxylases, whereas HIF-proline 4 hydroxylase-3 is more selective for the N-terminal proline [112]. Moreover, HIF-proline 4 hydroxylase-1 and HIF-proline 4 hydroxylase-3 appear to prefer HIF-2α over HIF-1α as a substrate [113].

As mentioned above, HIF-proline 4 hydroxylases also require ascorbate as a reducing factor. The concentrations of ascorbate allowing HIF prolyl hydroxylase regulation are in range of 140–300 μM [114], which is less than the millimolar cellular concentrations of ascorbate [115]. Further, while ascorbate appears to be particularly important for iron reduction, it was recently found that absence of cysteine is causing oxidative self-inactivation of HIF-proline 4 hydroxylase 1 (EglN1/PHD2) [116]. In line, ascorbate was dispensable for oxygen sensing in vitamin C synthesis-deficient mice and could be substituted by glutathione. In this context, it was described that a pair of cysteine residues in HIF-proline 4 hydroxylase 1 modulates its redox sensitivity [101].

Interestingly, treatment of endothelial cells with ascorbate reduced the thrombin-dependent HIF-1α and HIF-2α induction [117–119]. Similarly, treatment of cancer cells with ascorbate downregulated HIF-1α levels [120,121] via HIF hydroxylases [122]. Moreover, it was found that low-grade endometrial tumors presented higher ascorbate levels and low HIF-1α levels, while high-grade tumors with aggressive characteristics had lower ascorbate but high HIF-1α levels [123]. In line, when physiological levels of ascorbate were restored in mice deficient for endogenous vitamin C synthesis, tumor growth and HIF-1α activity were reduced' [124]. Similar characteristics were found in a lymphoma cell model in SCID mice where ascorbate supplementation reduced HIF-1α and tumor proliferation [125]. In relation to that, treatment of endothelial cells with ascorbate doses that were higher than 100 mg/dl suppressed capillary-like tube formation [126] that is usually stimulated by the HIF-target VEGF.

### 8.2. 2ODDs that control epigenetics

One of the first epigenetic changes discovered was the methylation of DNA bases. These methylations occur mainly as 5-methylcytosine (5 mC), but adenine residues can also be methylated.

These methylations also form the basis for further modifications, which are mediated by ten-eleven translocation proteins (TET). In this process, 5 mC is oxidized to 5-hydroxymethylcytosine (5hmC). In humans, three TET proteins are known to catalyze not only the conversion of 5 mC to 5hmC, but also the further conversion to 5-formylcytosine and 5-carboxylcytosine. The latter two can subsequently be eliminated via thymine DNA glycosylase-mediated base excision and the DNA base excision repair (BER) program, ultimately leading to incorporation of demethylated cytosines into DNA [127,128].

As with the HIF-proline 4 hydroxylases, ascorbate enhances TET catalytic activity. For example, ascorbate can globally increase the 5hmC content in mouse embryonic stem cells [100,129,130]. Similar effects were observed in colon, kidney, bladder, breast, and lung cancer cells as well as in human leukemia and hematopoietic stem cell (HSC) [131–137]. In line, ascorbate deprivation in L-gulonolactone oxidase-deficient mice impaired TET function and expanded the HSC compartment [100]. Likewise, from the three TETs, TET2 appeared to be of utmost importance for differentiation of HSCs in humans and L-gulonolactone oxidase-deficient mice. In those studies, it was found, that HSCs from mice with ascorbate deficiency and mice with TET2 deficiency lost their ability to properly differentiate and had more leukemia like features with excessive self-renewal [138–140]. The effects of ascorbate supplementation on TETs were not limited to HSCs as ascorbate also improved TET activity and reduced renal cancer cell growth [141,142]. In addition to its effects on the TET system, ascorbate also appeared to have TET-independent tumor-reducing effects, as this also occurred in cells capable of synthesizing ascorbate [139].

Hypoxia, similar to HIF-proline 4 hydroxylases, appears to decrease TET activity and 5hmC labeling on DNA; resulting in DNA hypermethylation in hypoxic tumor areas [143–145]. However, unlike HIF-proline 4 hydroxylases, TET enzymes seem not to act as oxygen sensors, as only oxygen concentrations below 2% $O_2$ resulted in TET

inhibition [143]. This, together with the relatively low $K_M$ value of 30 μM for $O_2$ confirms that TET activity is maintained over a wide range of oxygen concentrations [143]. Interestingly, there are also paradoxical effects, such as hypoxia mediated increases in TET expression and 5hmC marks in certain cell lines. This suggests cell type-specific compensatory mechanisms that depend on oxygen availability and TET levels [143]. The causes of these phenomena are not yet well understood, but such upregulation of TET proteins may be sufficient to prevent negative effects on DNA due to substrate and cofactor limitations.

In addition to modification of DNA bases, methylation of histones is another epigenetic modification. The discovery of histone demethylases (HDMs) showed that methylation of histones by histone methyltransferases (HMTs) is reversible and thus dynamic [146,147]. Histone methylation usually occurs at lysine (K) and/or arginine (R) residues. Histones H3 and H4 are most frequently affected.

Methylation of histones can be reversed by HDM histone demethylases. In principle, two histone Nε-methyllysine demethylase families (KDMs) can be distinguished. The first are the lysine-specific type demethylases (KDM1s or LSD) and the second are the Jumonji C domain-containing histone demethylases (JmjC KDM or JHDM). The latter belong to the 2ODD group and require $O_2$, $Fe^{2+}$, 2-oxolutarate, and ascorbate for their catalytic activity [148]; *i.e.*, to hydroxylate the methyl group of lysine. Furthermore, oxidative demethylation produces formaldehyde, succinate, and $CO_2$. The JmjC-KDMs act on all 3 Nε-methyllysine methylation states, whereas the KDM1s act only on di- and monomethylated histones [147,149].

Interestingly, ascorbate was found to cause a CpG demethylation in human pluripotent stem cells which coincided with increased expression of several KDMs such as KDM3A/JMJD1A, KDM3B/JMJD1B, JMJD1C, and KDM4B/JMJD2B [150]. Furthermore, the majority of the human JmjC KDM proteins were found to be inducible by hypoxia mainly via the HIF pathway (for review see Ref. [151] and references therein). Like with HIF-proline 4 hydroxylases, hypoxia, $H_2O_2$ or NO have been shown to inhibit histone demethylase activity [145,152]. Similarly, histone hypermethylation due to hypoxia has been widely described [151,153,154] and *e.g.*, increased H3K9me2/me3 and H3K36me3 levels in several promoter areas of chemokine encoding genes are indicative for this deficit in demethylase activity [155]. By contrast, increases in $Fe^{2+}$ and ascorbate rescued this inhibition [145,152]. In line with the multiple factors that can affect 2ODD apart from ascorbate, recent evidence indicated that several members of the JmjC KDM family, especially KDM5A, and KDM6A are able to act as oxygen sensors that can mediate cell differentiation in an HIF independent way [156,157].

Another family of 2ODDs involved in epigenetics are homologs of the *E. coli* DNA dealkylation enzyme AlkB. These can function as both DNA, and RNA demethylases which repair DNA and RNA bases damaged by S(N)2 alkylation reagents [158]. Those reagents attach hydrocarbons to endocyclic ring nitrogen atoms (N1 of adenine and guanine and N3 of thymine and cytosine). To date, nine AlkB homologs have been found in mammals and include ALKBH1-8 and fat mass and obesity associated protein (FTO) [158].

While the role of AlkB proteins in repairing DNA alkylation damage in prokaryotes has long been known, the functions of their nine homologs, particularly in mammals, became known essentially only after their structures were elucidated [158–161]. Although all Alk homologs have different specific substrates, what they have in common is that they hydroxylate a methyl group on the substrate, which is thereby first converted to an unstable hydroxymethyl intermediate that is subsequently released as formaldehyde. In the end, the demethylated substrate remains, which may include DNA or RNA bases as well as proteins.

ALKBH1 is found in the nucleus and in mitochondria and is particularly involved in the demethylation of 3-methylcytosine in mRNA [162,163], of 5-methylcytosine in mitochondrial tRNA, and of N1-methyladenine in tRNA [164] and N6-methyladenine in genomic DNA [165]. Further, in mitochondria ALKBH1 has been shown to produce 5-formylcytosine in the wobble position of methionine tRNA to expand codon recognition [166]. ALKBH2 and ALKBH3 mainly convert N1-methyladenine and N-methylcytosine in single-stranded and double-stranded DNA, and N1-methyladenine in mRNA. ALKBH2 is also able to repair adducts such as N3-ethylthymidine and etheno-adducts of DNA. ALKBH4 is involved in the demethylation of lysine 84 in actin, while ALKBH5 acts mainly on N6-methyladenine in mRNA [167] or N1-methyladenine in tRNA. The substrates for ALKBH6 and ALKBH7 are not yet known, but ALKBH8 methylates 5-carboxymethyluridine in the tRNA wobble position together with Trim112 to generate 5-methoxycarbonylmethyluridine [166,168,169]. Interestingly, FTO was shown to be transported between the cytoplasm and the nucleus, a process consistent with its action on N6-methyladenine in mRNA, which is first transcribed in the nucleus and then further processed in the cytoplasm [164].

## 9. Vitamin C and kinases

The nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) comprises a transcription factor protein complex that controls many cellular functions including cell growth, survival, and immune response often in response to various stimuli such as oxidative stress, irradiation, cytokines, heavy metals as well as bacterial and viral antigens. NF-kB levels may be enhanced or found with higher-than-normal activities in certain cancers. Similarly, increases in NF-kB may contribute to inflammatory disorders, such as ulcerative colitis, asthma, or rheumatoid arthritis.

The five NF-κB family members may form various dimers that when released from their inhibitors are able to bind to κB enhancer elements of target genes to activate transcription. The inhibitors are commonly part of a cytosolic complex that can be composed of either RELA, p50 and IkBα or RELB and p100. While the latter two proteins are crucial for the non-canonical NF-κB activation via NF-κB-inducing kinase (NIK), which induces phosphorylation-induced p100 processing, the canonical pathway depends on multiple extracellular signals that can activate the enzyme IκB kinase (IKK). Activated IKK catalytic subunits, mainly α, phosphorylate IκBα which results in its ubiquitination and subsequent proteasomal degradation.

Interestingly treatment of endothelial cells with vitamin C at concentrations that are found intracellularly in vivo, were reported to inhibit NF-kB activation triggered by e.g., IL1, or TNFα. Consequently, induction of a NF-kB -dependent gene expression was also inhibited [170,171]. Mechanistically, it was proposed that ascorbate scavenges free radicals generated during the stimulation and becomes oxidized into DHA. DHA, in turn, was found to act as a direct IKK inhibitor [172]. As a result, this shows that vitamin C can be engaged also in signal transduction by acting as kinase inhibitor.

In line, a recent nutrigenomic study found that vitamin C triggers extensive epigenomic and transcriptomic reprogramming in monocyte-derived dendritic cells. Thereby vitamin C caused extensive demethylation at NF-κB promoter sequences which was mediated by TET2. This was accompanied by enhanced antigen-presentation and an improved immune response [173].

## 10. Vitamin C and diseases

### 10.1. Common cold

For many, vitamin C is the remedy of choice to prevent an impending cold. If colds, coughs and the like have already set in, effervescent tablets or powders containing the vitamin are supposed to banish the annoying symptoms of the common cold again. The idea for this came from the Nobel Prize winner in chemistry, Linus Pauling. He began advertising oral intake of high vitamin C doses in the 1970s as protection against many different diseases - including the common cold [174]. Even today, many pharmaceutical manufacturers suggest that vitamin C is an effective remedy for colds. For example, it is found as an additive in medications that are supposed to combat typical symptoms such as fever

**Table 2**
Vitamin C, high dose - randomized controlled study results.

| Cancer | Patients | Control group | Intervention group | Endpoint | Results | Notes | Reference |
|---|---|---|---|---|---|---|---|
| Ovarian cancer stage III/IV, initial diagnosis | 27 | Paclitaxel/ Carboplatin | Paclitaxel/Carboplatin Vitamin C intravenously (75 or 100 g, twice weekly, for 12 weeks) | Adverse events Survival rate after 5 years | Reduction in chemotherapy-associated toxicity | The control group did not receive placebo No numbers of survivors reported | [219] |
| AML (>60y) | 73 | Decitabine, G-CSF, Cytarabine, Aclarubicin | Vitamin C 50–80 mg/kg day 0–9 Decitabine, G-CSF, cytarabine, aclarubicin | No primary endpoint stated | Significantly higher rate of complete remission after first induction Higher 3-year survival (15.3 vs 9.3 months) | (low-certainty evidence) | [220] |
| Metastatic colorectal cancer RAS mutation subgroup | 442 | FOLFOX+/ Bevacizumab | 1.5 g/kg body weight vitamin C intravenously on days 1–3 in addition to FOLFOX+/ bevacizumab every 2 weeks for 12 cycles | Progression-free survival (PFS) objective response rate (ORR) Overall survival (OS) | No significant differences in PFS, ORR and OS; Significant PFS prolongation in patients with RAS mutation and patients >55 years (9.2 vs 7.8 months) No major adverse effects | | [221] |

and headaches. In addition, the high-dose administration of vitamin C in connection with tumor diseases is attributed various effects such as cytotoxicity for malignant cells but not for healthy tissue, improvement of the quality of life of tumor patients, protection of healthy cells against chemotherapy-induced cytotoxicity, and effect enhancement of radiation therapy and, in certain cases, chemotherapy.

The stimulation of the body's defenses, which is often attributed to vitamin C, is explained in part by protection of the phagocyte membrane from oxidative self-destruction [175,176]. This oxidative self-destruction may otherwise be triggered by the myeloperoxidase triggered during phagocytosis (see above). In addition, increased interferon generation and activation of the complement system have been observed in animal studies after vitamin C administration [177]. In general, high ascorbic acid levels have been found in blood leukocytes, which play an important role in immune defense [178]. Furthermore, vitamin C appears to have an influence on numerous other neutrophil functions, such as chemotaxis, uptake of particles by phagocytes, lysozyme-influenced nonoxidative immune response, and stimulation of the hexose-monophosphate shunt [177].

However, the place of vitamin C administration in the control and prevention of diseases such as the common cold is scientifically quite controversially discussed [179–181]. Larger reviews and therapeutic studies see a general trend that vitamin C has no measurable prophylactic effect in seasonal colds in general [182,183]. However, under certain circumstances such as heavy acute physical stress, or particularly low dietary intake a moderate positive prophylactic effect as well as on duration and severity of symptoms could be observed [184–186]. Although these findings indicate that vitamin C is neither entirely preventing nor healing from common cold, its oral supplementation might be considered in individual patients to alleviate symptoms.

### *10.2. Cancer*

#### *10.2.1. Oral use of vitamin C*

With the widespread availability of vitamin C, numerous individual case reports as well as pilot studies were published that attested to a greater or lesser clinical benefit of oral vitamin C supplementation in tumor diseases [187]. The first two retrospective studies seemed to support these statements [188,189]. However, those studies were not properly randomized, which called their validity into question. Accordingly, the U.S. National Cancer Institute (NCI) commissioned two randomized, placebo-controlled, double-blind trials to evaluate the effect of oral vitamin C on symptoms and survival in patients with advanced malignancies [190,191].

In the first study participated 150 patients with various types of advanced cancer [190], and in the second study participated 101 people with advanced colorectal cancer [191]. In both studies, participants were randomly assigned to one of two groups. While the first group took 10 g of vitamin C per day orally, the second group took a placebo. Twenty-seven people in the vitamin group withdrew their consent and did not take their assigned medication. In the second study, this was true for one person. Thus, a total of 223 subjects' data were analyzed in both studies.

At the end of 10 months in the first study [190], and 39 months in the second [191], there was no difference in the number of survivors.

A third randomized-controlled trial [192] enrolled 30 women with breast cancer. While one group took oral vitamin C in addition to chemotherapy, the control group received only standard chemotherapy. The study did not investigate whether the participating women lived longer with vitamin C. Instead, in that study the authors only questioned whether taking vitamin C inhibits the progression of breast cancer. It could not provide evidence for this.

Overall, it seems that oral intake of vitamin C is of no benefit in the treatment of cancer and therefore, conventional oncology abandoned the use of vitamin C in tumor therapy in the 1980s [193,194].

#### *10.2.2. High dose intravenous use*

In the late 1990s, it was found that the concentrations of vitamin C in plasma and tissues are kept within narrow limits by intestinal absorption and renal reabsorption and excretion [195–197]. When oral intake of vitamin C exceeds 200 mg daily, it is impossible to increase ascorbate plasma and tissue levels further via oral administration. However, this is possible by intravenous administration, at least until physiological balance is restored by renal excretion. Thus, in healthy volunteers, oral administration of vitamin C at the highest tolerated dose of 3 g every 4 h resulted in a maximum plasma concentration of only 0.22 mmol/L. In contrast, an intravenous (i.v.) dose of 50 g of vitamin C resulted in plasma concentrations that reached a maximum of 13.4 mmoL/l [198]. Comparable results were also seen in tumor patients [199–201]. This knowledge, along with the increased understanding of ascorbate's function in oxygen sensing and epigenetics together with identification of different cancers with TET or JHDM mutations, especially hematological ones [202–204], has renewed interest in ascorbate in tumor therapies.

In mice with several aggressive tumor entities, daily application of high-dose intravenous vitamin C reduced tumor volumes in the range of 41–53% [205]. Reduced tumor growth was also observed in xenografted mice with human tumors and in human cancer cell lines [206–210].

Moreover, the effect of ascorbate in these studies might be based on mechanisms other than reactivation of 2ODDs, since the plasma

**Table 3**
Non-controlled vitamin C, high dose study results.

| Study design | Cancer | Patients | Control group | Intervention group | Endpoint | Results | Reference |
|---|---|---|---|---|---|---|---|
| No control group | different tumors, end stage | 24 | vitamin C intravenously (0.15–0.71 g/kg bw, daily, for up to 8 weeks, single treatment | Adverse events<br>Radiological imaging for tumor progression | intravenous vitamin C was well tolerated; 2 patients discontinued treatment, one patient's disease stabilized on treatment | doses used were low; plasma concentrations did not exceed 3.8 mM | [223] |
| No control group | different tumors, end stage | 39 | Vitamin C (10 g intravenously twice, and 4 g orally daily, for 1 week), only treatment | Quality of life | Patients had significantly lower scores on fatigue, nausea/vomiting, pain, and loss of appetite; other functions and symptoms were not significantly changed | Dosages used were low; very short treatment duration | [224] |
| Dose escalation, No control group | Different tumors or hematologic neoplasms that did not respond to standard therapy | 24 | Vitamin C intravenously (0.4–1.5 g/kg bw, three times weekly, for up to 30 weeks), single treatment | Adverse events<br>Pharmacokinetics | Intravenous vitamin C was well tolerated; pharmacokinetics and recommended doses determined; no objective tumor response | | [199] |
| Dose escalation, No control group | metastatic pancreatic cancer | 14 | Vitamin C intravenously (50, 75, or 100 g, three times weekly, for up to 8 weeks), in combination with gemcitabine and erlotinib | Adverse events<br>CT imaging for tumor response | Primary tumor regression; no adverse events other than symptoms of tumor progression and/or side effects of gemcitabine/erlotinib therapy | Short treatment duration | [225] |
| Dose escalation, No control group | Diverse solid tumors that did not respond to standard therapy | 17 | Vitamin C intravenously (30–130 g/m2, weekly for 4 consecutive days, for 4 weeks), as sole treatment | Adverse events<br>Quality of life<br>Pharmacokinetics | Intravenous vitamin C was well tolerated; pharmacokinetics and recommended doses were determined; no objective tumor response | | [226] |
| No control group | metastatic pancreatic cancer, histologically confirmed, stage IV | 9 | Vitamin C intravenously (15–125 g, twice weekly, for 69–556 days), in combination with gemcitabine | Adverse events<br>Time to progression and overall survival | Combination with gemcitabine well tolerated; evidence of statistically nonsignificant efficacy | evidence of statistically nonsignificant efficacy no sufficient patient numbers to evaluate efficacy | [227] |

concentrations achieved are far above the ascorbate values needed for HIF prolyl hydroxylases, which are reported to be between 100 and 300 μM.

As mentioned above, vitamin c is an antioxidant at low, physiological plasma concentrations (~0.1 mmoL/l), whereas it can be a prooxidant at high pharmacological concentrations (up to 20 mmoL/l). At the latter, for example, a high flux of hydrogen peroxide can alter redox homeostasis by depriving cells of reduced glutathione and promoting cytotoxic effects [49,211] such as damage to cell membranes and impairment of DNA and glucose metabolism. Furthermore, hydrogen peroxide seems to exert more potent toxic effects in tumor cells because antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase are present in low or unbalanced concentrations in most human tumor entities [50].

It is therefore possible that increased redox stress could contribute to the antitumor effect described in several case reports, in which patients with advanced tumors were treated with high-dose intravenous vitamin C [212–218]. In contrast to those case studies, only a few randomized controlled clinical trials tested whether high-dose vitamin C is effective against cancer and the available current evidence is low (Tables 2–4).

In the first study, a small randomized controlled phase I/IIa trial was conducted with 27 patients with newly diagnosed stage III/IV ovarian cancer. These patients were randomized to conventional paclitaxel/carboplatin therapy either alone (control group) or in combination with i.v. vitamin C (75–100 g; twice weekly for 12 weeks, treatment group) [219]. Although the authors reported a trend for improvement in 5-year survival, there were no numerical data provided and 27 participants are far too few for a reliable statement. The probability is therefore high that the results are merely based on random fluctuations and that in reality vitamin C is not acting against ovarian cancer in those patients. However, it was shown that the additional intravenous administration of high-dose vitamin C reduced chemotherapy-related toxicities.

Another randomized trial evaluated the efficacy of intravenous vitamin C on remission rates and overall survival in 73 patients aged 60 years and older with acute myeloid leukemia (AML) receiving chemotherapy [220]. During chemotherapy, the control group received decitabine, cytarabine, aclarubicin, and G-CSF whereas the intervention group received additional i.v. vitamin C (50–80 mg/kg on day 0–9). The authors reported a significantly higher rate of complete remission in the intervention group after the first chemotherapy induction and a similar trend after the second induction. Furthermore, there was also a significantly higher 3-year survival rate in the vitamin C group at 15.3 versus 9.3 months in the control group, however, there was low certainty evidence [220].

In the largest randomized, multicenter phase III study to date, the i.v. administration of vitamin C in addition to chemotherapy was investigated in 442 patients with metastatic colorectal cancer (mCRC). As chemotherapy the patients control group received FOLFOX+/bevacizumab and the intervention group received in addition to chemotherapy vitamin C (1.5 g/kg i.v.) on days 1–3 of the chemotherapy cycle. The cycle was repeated every 2 weeks and ended after a maximum of 12 cycles. The progression-free and overall survival based on radiological response (RECIST) was measured [221]. Compared with the control group, there were no significant differences in progression-free survival, overall response rate, and overall survival. However, in a subgroup of patients that were older than 55 years and where tumors displayed RAS mutation, progression-free survival was significantly prolonged compared with the control group (9.2 vs. 7.8 months, $p = 0{,}01$). Overall survival was not improved in this subgroup, showing only a trend. However, the authors believe that the true effect of the vitamin C infusion is underestimated, because it was administered only every fourteen days and ended after six months before most of the disease progression had occurred [221].

For healthy individuals, high-dose intravenous vitamin C is generally unproblematic. The main side effects during infusion are thirst and increased urine flow. Adverse events following the intravenous

**Table 4**
Vitamin C, high dose retrospective studies results.

| Study design | Cancer | Patients | Control | Intervention | Endpoint | Results | Notes | Reference |
|---|---|---|---|---|---|---|---|---|
| controlled, retrospective | end-stage tumor patients | 100 | historical control group (n = 1.000) | Vitamin C intravenously (10 g daily for 10 days, then orally; or orally only) as sole treatment | Overall survival | 4-fold increase in median survival in vitamin C group | some patients received vitamin C orally rather than intravenously; short treatment duration; dosage quite low | [189] |
| controlled, retrospective | end-stage tumor patients | 100 | historical control group (n = 1.000) | Vitamin C intravenously (10 g daily for 10 days, then orally; or orally only) as sole treatment | Overall survival | 5-fold prolongation of median survival in vitamin C group | some patients received vitamin C orally rather than intravenously; short treatment duration; dosage quite low | [228] |
| controlled, retrospective | end-stage tumor patients | 294 | historical control group (n = 1.532) | Vitamin C intravenously (10 g daily for 10 days, then orally; or orally only) as the only treatment | Overall survival | 2-fold prolongation of median survival in the vitamin C group | some patients received vitamin C orally rather than intravenously; short treatment duration; dosage quite low | [229] |
| Epidemiologic, retrospective cohort study | Breast cancer | 53 | breast carcinoma (n = 72) | vitamin C intravenously (7.5 g, once weekly, for ≥4 weeks) in combination with standard therapy | Adverse events | intravenous vitamin C was well tolerated; improvement in quality of life; no effect on tumor status | the doses used were low | [230] |
| No control group, retrospective | different tumors, after cessation of conventional tumor therapy | 45 | | intravenous vitamin C (7.5–50 g, 1–100 treatments) as the only treatment | CRP Inflammatory parameters Tumor marker proinflammatory cytokines | modulation of inflammation correlates with decreases in tumor markers | | [231] |

administration of vitamin C are minor and correspond to the side effects to be expected with rapid infusion of highly osmolar solutions. In isolated cases, nausea/vomiting, chills, and/or headache may occur. After infusion, some people report drowsiness or leg edema, which may last for several days. They can be avoided by drinking fluids before and during infusion [199,201,222]. Caution should be exercised in patients with a tendency to kidney stones, with renal insufficiency, and with erythrocytic glucose-6-phosphate dehydrogenase deficiency; Patients with iron storage disorders should not receive high-dose vitamin C at all.

Overall, the results of the randomized controlled trials suggest (partly in subgroup analyses) that high-dose intravenous vitamin C administered in addition to chemotherapy could have an antineoplastic effect in patients with advanced ovarian cancer, AML of advanced age (>60y.), metastatic colorectal cancer of advanced age (>55y.) and, independent of age, with RAS mutation.

## 11. Conclusion

Vitamin C is undoubtedly necessary for the maintenance of the general homeostasis of cells and tissues. This becomes mechanistically visible mainly through its role as a reducing agent, which contributes in particular to the maintenance of the full functionality of a number of enzymes. However, the focus on the antioxidant properties of ascorbate has largely ignored the potential prooxidant effects and associated functional changes.

The data from high dose intravenous ascorbate clinical trials suggest that more trials and research as well as clinical insight is required to reach a concise view. For example, it is necessary to identify those cancer patients that would benefit from high dose i.v. ascorbate supplementation. Further, it may well be that different cancers would require different dosing regiments, hence, finding the optimal level of ascorbate supplementations is necessary. Thereby, new findings obtained by in-depth research on vitamin C transporters, functional impairments and possible interactions/competitions with glucose and other molecules could be of benefit. The latter may be especially important in cancers that are based on mutations in ascorbate requiring enzymes such as 2ODDs as seen in leukemias with JHDM or TET2 mutations.

Further studies, especially controlled randomized clinical trials as well as on research on appropriate in vivo models, are needed to assess the actual association between vitamin C, cancer risk and incidence as well as the pathogenesis of various stress-induced diseases in the context of altered nutrition.

## Declaration of generative AI and AI-assisted technologies in the writing process

The author has nothing to disclose.

## Declaration of competing interest

The authors declare that there is no conflict of interest.

## Data availability

No data was used for the research described in the article.

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