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Vitamin C – A Wonder Drug from Nature's Own Pharmacopeia?

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This review outlays the biological chemistry of vitamin C (ascorbic acid) that explains its increasing therapeutic use in orthomolecular medicine. Even in mega doses that are often mostly and safely administered as intravenous infusions, the vitamin, as such or in its potent transportable form dehydroascorbic acid, does not affect healthy cells and targets only cancerous cells. The review proposes the need for clinicians in mainstream medicine to explore more intimately vitamin C's integrative use in treating many modern-day health problems, such as non-hormone dependent cancers, diabetes, Alzheimer's disease and sepsis, based on a proper appreciation of the strengths and limitations of both RCTs (efficacy studies) and observational studies (effectiveness studies). Vitamin C is a first line antioxidant. Its known pro-oxidant effects in killing cancer cells, along with its ubiquitous roles as an enzyme cofactor, offer much potential to be tapped in cancer therapy, either alone or in combination with other therapies. An adequate intake of dietary and supplemental vitamin C (90-120mg/day), is seen as a pivotal need for both young and old to ward off a plethora of health problems.

Keywords: L-ascorbic acid; first line antioxidant; essential enzyme cofactor; immune system booster; essential dietary supplement; Vitamin C treatment in human disease; IV-Vitamin C

I. INTRODUCTION

Vitamin C or ascorbic acid is a simple organic molecule that belies its potency in nutrition and health. The body does not produce it, and humans require a daily intake of food that contains it. It is an essential micronutrient for the growth, development and repair of all body tissues, and a cofactor for a family of biosynthetic and gene regulatory enzymes. It is a highly effective antioxidant, due to its ability to readily donate electrons, thus protecting important biomolecules from free radical damage.

Vitamin C first came into prominence in the 18th century in the treatment of scurvy affecting severely undernourished sailors. Patients stricken with the disease suffer from weakening of collagenous structures, which results in poor wound healing, and impaired immunity. A massive build-up of histamine occurs in the bodies of such patients on account of low levels of vitamin C, which in turn results in reduced levels of the histamine metabolising enzyme, diamine oxidase. However, it was Linus Pauling, a double Nobel Laureate in chemistry, who in the 1970s famously and controversially claimed that consumption of the vitamin in mega doses can confer extraordinary health benefits ranging from the prevention and treatment of the common cold to other ailments such as cancer - a prescription whose plausibility rested on the firm grounds of the vitamin's well-known water solubility and excretion in urine, and its non-toxicity to healthy cells. Was he right? The question has taken on some new relevance in the light of the findings of a recent study (Brasky *et al.*, 2017) involving a large VITAL (vitamins and lifestyle) cohort which showed that heavy supplementation of vitamins B6 and B12 was associated with a 30-40% increase in lung cancer risk among men.

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In the case of the common cold, it would seem that the overwhelming consensus among the scientific community today after many trials and data analysis is that vitamin C offers no benefits or protection against it even at mega doses (50 -200g/day) administered intravenously; at best it only helps to relieve cold and flu symptoms (Gorton & Jarvis 1999; Hemilä & Chalker 2013). The incidence and severity of the cold remain unaffected, meaning the prophylactic intake of vitamin C is bereft of any clinical effects.

The jury, on the other hand, is still out on the vitamin's neoadjuvant and adjuvant roles in cancer prevention and treatment, particularly of non-hormone dependent cancers. Block in his 1991 review created much optimism by presenting strong epidemiologic evidence for the protective effects of high intake vitamin C against cancers of the oesophagus, larynx, pancreas, stomach, breast and cervix, quoting studies in which a dietary vitamin C index was calculated.

However, such observational studies are seldom heeded by clinicians who are guided in their practice by evidence-based medicine, and who regard randomised clinical trials (RCTs) as the 'gold standard' to evaluate the efficacy of therapy or an intervention intended to improve outcome. Faraoni and Schaefer (2016) have argued that it is important to understand the strengths and limitations of both RCTs (efficacy studies) and observational studies (effectiveness studies) and that none of the study designs should be considered in isolation. They contend that *"interpretation of the results obtained from both RCTs and observational studies should be made to help understand the efficacy/effectiveness and safety of a therapeutic option"*. This is particularly relevant when considering nutritional matters since there can be several reasons why the results of observational studies and RCTs of nutritional agents might not be in accord, particularly when RCTs are null. Most prominent among these is the selection of an inappropriate intervention dose (too high or too low), duration (too short), timing (too late in life), or study population (already has adequate nutrition) (Kristal 2008).

The observational approach, however, appears to be the mainstay of investigative studies in orthomolecular medicine (Levy 2002), with the findings being often unreservedly accepted by practitioners of complementary and alternative medicine. The therapeutic potential of vitamin C has gained much credibility in their hands. They have found high doses of vitamin C (often via intravenous infusions) to be a panacea for treating all forms of health disorders. Other than cancer, these include heart disease, blood pressure, cataract and age-related macular degeneration, dental cavities and plaque, constipation, Lyme disease, human immunodeficiency virus (HIV) disease, stomach ulcers caused by *Helicobacter pylori*, collagen disorders, depression, dementia, Alzheimer's disease, fatigue including chronic fatigue syndrome (CFS), autism, schizophrenia, gout, Parkinson's disease, back pain and disc swelling, osteoporosis and other bone conditions, acne and other skin conditions, and iron deficiency. The effectiveness of vitamin C in epidemiological studies bearing on some of such diseases is briefly examined in this review.

II. VITAMIN C BASICS

A. Exogenous source, Biosynthesis and Molecular Structure of Vitamin C

Vitamin C is abundant in plants and fruits, and most animals, but is conspicuously absent in humans due to a non-functional gene for the enzyme required for its biosynthesis, namely, L-gulonolactone oxidase. This enzyme is essential for the synthesis of 2-keto-l-gulonolactone, its direct precursor. Adequate amounts of vitamin C has thus to be secured from the diet. In solution, vitamin C exists in two forms; the reduced form, L-ascorbic acid (ascorbic acid, ascorbate, AA), and the oxidised form, dehydro-L-ascorbic acid (dehydroascorbic acid, DHA). Vitamin C is transported in mammalian cells by two types of proteins: sodium-ascorbate co-transporters (SVCTs) and hexose transporters (GLUTs). The SVCTs actively import AA, while GLUTs mediate the transport of DHA, which allows for its recycling, and perhaps accounts for the low daily requirements of vitamin C in humans.

Generally, the plasma concentration of <11μM of vitamin C is considered to be deficient, 11–28μM is depleted or marginally deficient, 28–40μM is adequate, and >40μM is optimal. However, the body has some ability to overcome the deficiency of the vitamin by finely regulating the activation of HIF-1α, an oxygen-sensitive subunit of the heterodimeric HIF-1 transcription factor, whose expression is induced under hypoxic conditions. With sufficient supply

of vitamin C, the HIF transcription factor is less active than in conditions of vitamin C deficiency. Vitamin C is a critical cofactor for oxygen-sensing hydroxylases to down-regulate HIF-1. This raises the question of whether overexpression of HIF-1 induced by vitamin C deficiency could indirectly promote tumour growth since hypoxia is common in many types of solid tumours where rapid proliferation leads to obstruction and compression of blood vessels. Many cancer biologists view this to be a distinct possibility and have directed their research to find therapeutic strategies that inhibit HIF-1α pathway in cancer cells (Masoud & Li 2015).

It is equally pertinent here to ask whether raising levels of tumour ascorbate could slow tumour growth by moderating HIF-1 activation. Indications are that it does, and this may well prove to be an alternative mechanism for its role in preventing cancer growth rather than by mere radical scavenging. Indeed, recent pre-clinical studies with tumour tissue from cancer patients have shown a correlation between cellular ascorbate levels, HIF-1 activity, tumour size and patient outcome in breast, colorectal, endometrial and renal cancers (Carr 2017).

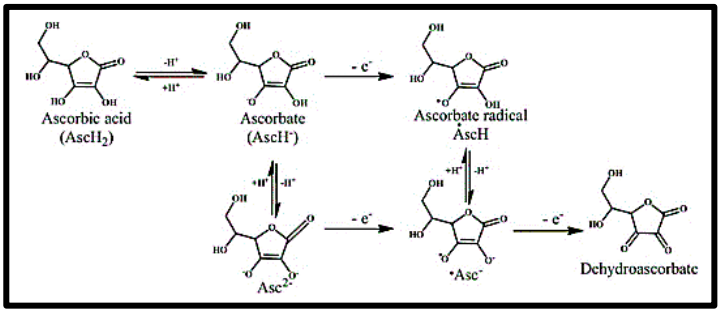
B. Redox Metabolism and Antioxidant Action of Vitamin C

Vitamin C is a potent water-soluble antioxidant that functions both intracellularly and extracellularly. In many tissues, vitamin C is present in millimolar concentrations. This helps to prevent intracellular protein oxidation (Stadtman & Berlett 1997). Tissues that fall into this category are those with high oxidant production and/or oxygen concentration such as lung, eye tissues exposed to light, monocytes, neutrophils and macrophages (Padayatty *et al.*, 2003).

In vitro studies suggest that vitamin C is the principal antioxidant in plasma for quenching aqueous peroxyl radicals as well as lipid peroxidation products (Polidori *et al.*, 2004). In addition, vitamin C recycles other antioxidants, including α-tocopherol (vitamin E) and tetrahydrobiopterin (THB). THB plays a critical role in the function of endothelial nitric oxide synthase (eNOS). Vitamin C deficiency results in the incomplete regeneration of THB resulting in the uncoupling of eNOS and the generation of the destructive superoxide and peroxynitrite radicals (May & Harrison 2013).

The vitamin loses electrons sequentially, with ascorbyl radical as an intermediate. In comparison with other free radicals, the ascorbyl radical is relatively stable and unreactive, and reacts poorly with oxygen, producing little if any superoxide. Dismutation of two molecules of the ascorbyl radical leads to the production of one molecule of ascorbic acid (AA) and one molecule of the stable dehydroascorbate. The dehydroascorbate (DHA) does not have any antioxidant capacity and is converted back into AA by the action of several glutathione-dependent DHA reductases, such as glutaredoxin (which also functions as a thioltransferase), protein-disulphide isomerase and glutathione *S*-transferase omega (Vera *et al.*, 1993; Wells & Xu 1994). It is also conceivable for the DHA to be converted back to AA by NADPH-dependent reduction mediated by thioredoxin reductase or by 3*α-hydroxysteroid dehydrogenase (May & Li 2003)*. DHA exists as a dimer in the crystalline form, but it spontaneously converts to a hydrated monomer in aqueous solution.

The mechanism of radical scavenging by vitamin C is shown in scheme 1 (Nimse & Pal 2015).



Scheme 1. Mechanism of antioxidant action of vitamin C

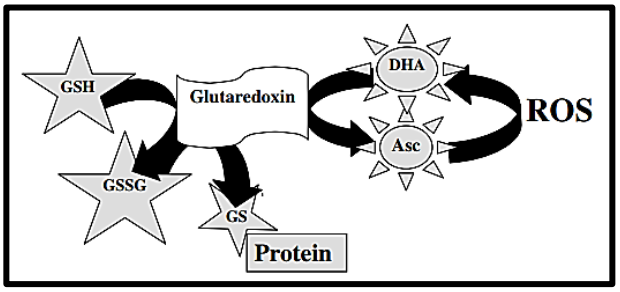
Source: Nimse & Pal 2015; CC BY 3.0

While AA is present in much higher concentrations in the serum, intracellular transport is limited only to some tissues. This is not so with DHA which has a broader distribution via the glucose transporters GLUT. After transport, DHA is reduced to AA and is trapped intracellularly (Vera *et al.*, 1993), which provides for the rapid and durable high concentration levels of AA needed to mitigate the effects of ROS.

Several animal model studies on rats (Ehrhart & Zeevalk 2003) and an in vitro study (Montecinos *et al.*, 2007) on oxidatively stressed human endothelial cells have shown that in its antioxidant action vitamin C has a functional interdependence on glutathione. Glutathione, a non-protein thiol, is also a water-soluble antioxidant like vitamin C. It is found predominantly as reduced glutathione (GSH) with deficient levels of oxidised glutathione (GSSG) under physiological conditions. These studies have confirmed that despite its ten-fold excess intracellular concentration over vitamin C, the presence of both antioxidants is required for full antioxidant protection. That vitamin C, in turn, is required to maintain cellular levels of glutathione (glutathione sparing effect) have come from a prospective nutritional study (Waly *et al.*, 2015) that investigated 200 healthy young adults with adequate (ADI) and low (LDI) dietary intakes of vitamin C. The subjects were tested for fasting plasma levels of the vitamin, serum antioxidant parameters [glutathione (GSH), thiols, and total antioxidant capacity (TAC)], and oxidative stress markers [malondialdehyde (MDA), and nitrites plus nitrates (NN)]. Oxidative stress in the sera of the LDI group was evidenced by depletion of GSH, low thiols levels, impairment of TAC, an elevation of MDA, and increased NN. In the ADI group, positive correlations were found between plasma vitamin C and serum antioxidant parameters (GSH, thiols, and TAC). Plasma vitamin C correlated negatively with serum MDA and NN levels in the study.

A catalytic mechanism for the cooperative interaction between ascorbate and glutathione-mediated by glutaredoxin has been postulated (Scheme 2) by Ehrhart & Zeevalk (2003).

In this scheme, glutaredoxin using GSH as an electron donor reduces DHA back to ascorbate and the resulting GSSG acts as a substrate for the thioltransferase activity of glutaredoxin to glutothionylate protein (Pr-SSG). Thus, the coupling of these reactions not only maintains reduced ascorbate for further ROS removal, but also removes intracellular GSSG and subsequently regulates the GSSG/GSH redox pair. It also explains why stimulation of Pr-SSG by ascorbate is only observed when there is oxidative stress.



Scheme 2. Glutaredoxin-coupled 'ascorbate–GSH cycle'
Source: Ehrhart & Zeevalk 2003; *Reproduced with permission.*

There are, however, two points to note concerning the antioxidant role of vitamin C.

The first of these is that an antioxidant can also behave as a pro-oxidant. Vitamin C has been demonstrated to act as a pro-oxidant *in vitro*, especially in the presence of transition metals such as iron and copper, whose reduced forms generate free radicals through the Fenton reaction.

$$2Fe^{3+}+AA \rightarrow 2Fe^{2+}+DHA;$$

$$2Fe^{2+}+2H_2O_2 \rightarrow 2Fe^{3+}+2OH\bullet+2OH^-$$

Such radicals can exert a damaging effect on DNA and other macromolecules in the body. Although the pro-oxidant activity of the vitamin remains to be established *in vivo*, it is prudent that vitamin C supplements be not recommended in people with high iron levels.

The second point to note is that AA is a reducing agent which exists in the body in reversible equilibrium with its oxidation product, DHA. This suggests that the effect of vitamin C in influencing disease states probably needs to be considered in the context of this partnership. It would be a hugely desirable study to probe deeply into the metabolic functions of this partnership, especially about the maintenance of optimum 'redox' conditions in tissues. Some

initial efforts in the clinical application of DHA have recently been reported (*vide infra*).

C. Enzyme Cofactor roles of Vitamin C

Vitamin C is a cofactor for eight different enzymes that are either monooxygenases or dioxygenases. It is involved with collagen synthesis, carnitine synthesis, regulation of DNA and histone methylation, proteoglycan deglycanation, converting dopamine to noradrenaline, and hormonal regulation, to name a few. The adrenal gland is among the organs with the highest concentration of vitamin C in the body. Ascorbic acid is a cofactor required both in catecholamine biosynthesis and in adrenal steroidogenesis (Patak *et al.*, 2004). Recent progress in the epigenetics field has identified a number of dioxygenases which catalyse the epigenetic modifications of DNA and histone; some of them require ascorbate as a cofactor (Monfort & Wutz 2013). This implies an ascorbate influence on the epigenome, and thus an impact on health and diseases. That the vitamin, indeed, plays an important and novel role as an epigenetic anticancer agent has come from recent studies (Cimmino *et al.*, 2018) demonstrating a high-dose intravenous vitamin C (IVC) therapeutic effect in TET2 (Tet methylcytosine dioxygenase enzyme)-mutant myelodysplastic syndrome, which are cancers in which immature blood cells in the bone marrow do not mature and, therefore, do not become healthy blood cells. Fig 1 summarises some of such cofactor roles of vitamin C.

Collagen makes up 70% of cartilage and up to 90% of tendons and ligaments. It plays an important role not only in bone health but also in vein and artery health and optimal brain function. Together with another protein called elastin, it makes up the composition of connective tissues in the body. A primary function of Vitamin C in the body is maintaining collagen levels. Vitamin C plays a vital role in healing wounds and burns. This is because it facilitates the formation of connective tissue in the actual scar. Connective tissue diseases come in different types and are mostly inherited. Among them, the most common is rheumatoid arthritis. In this systemic disorder, immune cells attack and inflame the membrane around joints. It can also affect the heart, lungs, and eyes. Intravenous vitamin C infusion in the dosage range 7.5 to 50 g/day has been demonstrated (Mikirova *et al.*, 2012b) to reduce inflammation and pain levels as judged by decreases in the measurements of the inflammation marker CRP. The decrease on average was by 44%, and the effect of the treatment was IV-C frequency dependent.

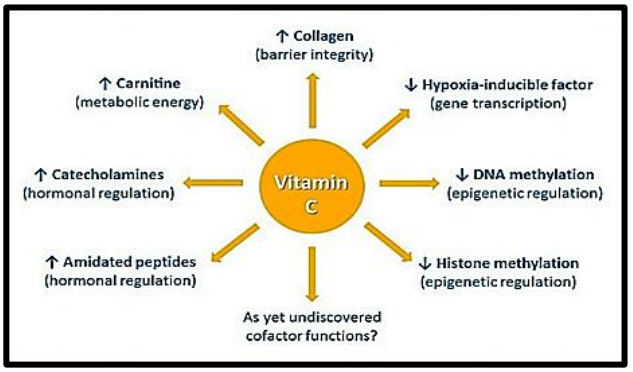


Figure 1. Cofactor roles of vitamin C in the body

Source: Carr & Maggini 2017; CC BY 4.0.

Carnitine plays a critical role in energy production. It transports long-chain fatty acids into the mitochondria for adenosine triphosphate (ATP) synthesis.

New roles for vitamin C have also been uncovered in the regulation of gene transcription and cell signalling pathways through regulation of transcription factor activity and epigenetic marks (Englard & Seifter 1986; Young *et al.*, 2015).

D. The Immune System and Vitamin C

While vitamin C's role in wound healing and tissue maintenance is well appreciated, an overlooked strength is its impact on boosting immune function**.**

There is much-established evidence to show that vitamin C is a crucial player in various aspects of the immune system (Carr & Maggini 2017)· Several cells of the immune system need it to perform their task, especially phagocytes and T-cells. Vitamin C stimulates several immune functions, such as cell motility and phagocytosis, resistance to oxidative damage, and interferon release. It is also needed for apoptosis and clearance of the spent neutrophils from sites of infection by macrophages, thereby decreasing necrosis/NETosis (a unique form of cell death that is characterised by the release of de-condensed chromatin and

granular contents to the extracellular space) and potential tissue damage. The role of vitamin C in lymphocytes is less clear, but it has been shown to enhance differentiation and proliferation of B- and T-cells, probably on account of its gene-regulating effects.

It is well documented that ageing individuals tend to have lower levels of vitamin C circulating in their bloodstream and immune cells. This can lead to impaired immune function. The recent availability of liposome-encapsulated vitamin C has aroused much interest as an alternative to IVC infusions for administering high levels of the vitamin that may be required. The predominant uptake of this form of vitamin C appears to be by the lymphatic system in the gut, which has the effect of loading up the immune cells in the lymphatics (Levy 2014). Although the amount that finally reaches the bloodstream is thus lessened, it is believed that almost all that appears in the blood readily enter other cells in the body because of better lipid penetrability. In as much as oral un-encapsulated vitamin C intake has only a 20% absorption rate into the bloodstream, a well-formulated liposomal vitamin C, such as with phosphatidylcholine, for example, as the carrier, would conceivably favour better passage of the vitamin through the digestive system. Clinical trials in humans using encapsulated vitamin C, however, are yet to be initiated.

III. VITAMIN C IN HUMAN DISEASE

A. Cancer and Vitamin C

Among the cancers studied in probing the therapeutic effects of vitamin C, breast cancer has perhaps received the most attention. The results to-hand, however, are mixed. A meta-analysis study conducted by the Karolinska Institute in Sweden with data from ten observational studies involving over 17,500 women patients revealed that high oral intake of vitamin C supplements significantly improved their chances of survival; a 22% reduction in mortality was observed (Harris *et al.*, 2014).

The mechanism of action of the vitamin on cancer cells was not defined, but it was proposed that in mega doses the vitamin exhibits a pro-oxidant activity arising from its routine antioxidant property that generates reactive free radicals. A systematic review by Putchala *et al.*, (2013) indicates that the pro-oxidant activity of pharmacological ascorbic acid is a part of its dose-dependent bimodal activity and is a result of the Fenton mechanism. The pro-oxidant role was evidenced for the vitamin by these researchers against tumours of the oral cavity.

A dramatic alteration in the metabolomic profiles of two human cancer cell lines – MCF7 (breast) and HT29 (colon) – following treatment with IVC was reported by Uetaki *et al.*, (2015). These researchers showed that for both cell lines, the levels of upstream metabolites in the glycolysis pathway and TCA cycle were increased, while ATP levels and adenylate energy changes suffered a concentration-dependent decrease. These results attest to vitamin C-induced oxidative stress in the cancer cells.

In a related translational study (McConnell *et al.*, 2013) oral administration of high doses of the vitamin was shown to improve the efficacy of chemotherapy in some patients, but not in all or with all combination therapies. However, intravenous infusion of vitamin C has more consistently helped improve outcomes for cancer patients by modulating inflammation, and with reduced side effects (Mikirova 2014). Chronic inflammation, if not contained, allows mutant cells to develop into fast-growing cancerous tumours.

The intravenous administration of high dose vitamin C (IVC), typically at an average dose of 0.5g/kg, is generally well tolerated, although some patients experienced transient adverse events during or after IVC infusions. Pre- and post-chemotherapy pharmacokinetic profiles show that tissue uptake of vitamin C increases after chemotherapy, with no increase in urinary oxalic acid excretion (Mikirova 2014). Plasma concentrations of roughly 30mM appear sufficient for preferential cytotoxicity against cancer cells (Casciari *et al.*, 2001).

More recently, the selective toxicity of vitamin C at mega doses to cancer cells has been demonstrated and explained by Iowa University researchers (Doskey *et al.*, 2016) who have administered the vitamin intravenously to rats and limitedly in phase 1 trials to humans afflicted by pancreatic or lung cancers. As opposed to oral administration, the intravenous route created blood levels of the ascorbate that were 100- 500 times higher. The researchers established that the mode of action of vitamin C in mediating cancer cell death was through *in vivo* generation of hydrogen peroxide

in the extracellular environment of tumour cells. Hydrogen peroxide can severely damage tissue and DNA, but healthy cells have several ways to keep it at deficient levels, so it does not cause damage. The ubiquitous enzyme catalase plays a central role in this. The researchers discovered that cells with low levels of catalase activity were more susceptible to damage and death when they were exposed to high doses of vitamin C.

However, the widespread reports in orthomolecular medical journals on the positive therapeutic effects of vitamin C at concentrations insufficient to induce direct tumour cell death strongly suggest the possibility that the vitamin itself may be exerting anti-angiogenic effects. This hypothesis has been tested and confirmed by Mikirova and co-workers (2008) at the Riordan Clinic in Kansas who showed that 25–60g of the vitamin affected the functions of both endothelial progenitor cells (HUAEC) and mature endothelial cells (HUVEC) involved in the process of angiogenesis. Their conclusion was based on several experimental investigations – the ability of both cell lines to migrate toward the angiogenic stimulus released from tumour cells, to proliferate to provide the necessary number of cells for making new blood vessels and to form capillary tubes, and on the nitric oxide production assay within endothelial cells. Their results are in support of an earlier study (Telang *et al.*, 2007) which showed that depletion of ascorbic acid powerfully restricts tumour growth *in vivo* in mice. These authors have noted from immuno-histochemical analyses of tumours that HIF-1*α* expression was not significantly altered by the absence of ascorbic acid, and have postulated that inhibition of tumour growth probably arises from interference with the process of angiogenesis which requires that stabilised type IV collagen be deposited by endothelial cells into the basement membrane of new blood vessels. The presence of AA is necessary to keep the iron-containing enzyme prolyl hydroxylase in its reduced state for effecting the hydroxylation of proline that contributes to the stability of the type IV collagen.

With regard to stomach cancers, particularly gastric adenocarcinoma (GA) and oesophageal squamous cell carcinoma (ESCC), the most recent investigation is a cohort study by Chinese scientists (Lam *et al.*, 2013) that also included a meta-analysis of the literature up to that period. The researchers found an inverse association between higher pre-diagnostic concentrations of plasma vitamin C and subsequent risk of incident GA in the test population, but there was little evidence of relation with risk of incident ESCC.

In respect of GA, the risk is potentiated if *Helicobacter pylori* bacteria are present in the stomach. *H. pylori* can induce a pre-cancerous condition known as atrophic gastritis, which can lead to increased gastric juice pH that impairs vitamin C secretion from the gastric mucosa. It is the leading risk factor for GA. Eradication of *H. pylori* and following normalisation of ascorbate levels in the gastric juice appear to critical in the control of GA (Aditi & Graham 2012). Diets rich in vitamin C appear to offer some protection from the development of atrophic gastritis. This is a consequence of the potent antioxidant properties of the vitamin which scavenges reactive mucosal oxygen radicals caused by *H. pylori*, and may even inhibit the growth of these bacteria, although evidence for the latter is equivocal **(**Zhang & Farthing 2005; Drake *et al.*, 1996).

Findings from another cohort study (Xu *et al.*, 2015) have shown that dietary exposure to nitrate, a precursor of N-nitroso compounds (NOC), may increase the risk of GA among individuals without a history of *H. pylori* infection. *In vivo* formation of NOC in the stomach occurs by chemical and bacterial nitrosation.

Ascorbic acid may reduce the risk of GA by inhibiting gastric nitrosation. This has been substantiated by measurements of ascorbic acid levels in gastric juice which are often found to be higher than in plasma, except in individuals where the levels are initially low and in whom gastric pathology has affected secretion (Schorah *et al.*, 1991).

In the case of oesophageal cancers, oxidative damage from factors such as gastroesophageal stress, smoking which causes inflammation, esophagitis, and increased cell turnover, may initiate the carcinogenic process (Mirvish 1995). In as much as NOC has also been implicated in the aetiology of oesophageal and other (e.g. bladder and nasopharyngeal) cancers, a protective role for antioxidants such as vitamin C can be envisaged.

An unexpected mechanistic rationale for exploring the

therapeutic use of vitamin C has come from the serendipitous observation by Yun *et al.* (2015) working on mice infected with cultured human colorectal cancers harbouring KRAS or BRAF mutations. The mutated cells make unusually large amounts of GLUT-1, the glucose transporter protein to survive. This protein also transports the oxidised form of vitamin C, dehydroascorbic acid (DHA), into the cell. Increased DHA uptake causes oxidative stress as intracellular DHA is reduced to vitamin C, depleting glutathione. Thus, reactive oxygen species accumulate and inactivate glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Inhibition of GAPDH in highly glycolytic KRAS or BRAF mutant cells leads to an energy crisis and cell death not seen in KRAS and BRAF wild-type cells. Tumour growth in the infected mutant mice fed with high doses of vitamin C was impaired relative to control. The results have opened up a new frontier for IVC for treating human colorectal cancers with KRAS or BRAF mutations.

Inflammation and elevated C-reactive protein (CRP) are associated with poor prognosis and decreased survival in many types of cancer. A pilot study reported by Mikirova *et al.* (2012, 2013) has revealed that a high dose of IVC (7.5g - 50g) leads to a significant suppression of the levels of inflammation cytokines IL-1α, IL-2, IL-8, TNF-α, eotaxin and CRP in the serum or heparin plasma of patients with prostate cancer, breast cancer, bladder cancer, pancreatic cancer, lung cancer, thyroid cancer, skin cancer and B-cell lymphoma who were administered the vitamin following treatments with conventional methods. The decreases in CRP also correlated with decreases in the tumour markers (PSA, CEA, CA27.29 and CA15-3).

The researchers, however, note that high inflammation or tumour burdens (among other factors), as measured respectively by CRP and tumour antigen levels, also tend to lower peak plasma ascorbate levels after IVC. When compared to patients with localised tumours, patients with metastatic tumours tend to achieve lower post infusion plasma ascorbate concentrations. However, as IVC can modulate inflammation, this, in turn, might improve outcomes for cancer patients.

While many examples attest to chronic inflammation either driving or paving the road for malignant transformations (Afrasiabi *et al.*, 2015), the mechanisms by which inflammation contributes to metastasis remain poorly understood.

Based on insights drawn from clinical pharmacokinetics and in vitro cancer-specific cytotoxicity of vitamin C, intravenously administered vitamin C in high doses (0.3-20 mM) appears worthy of clinical investigation in cancer therapy (Padayatty *et al.*, 2006, Jacobs *et al.*, 2015). Much of the reports to-date are based on observational studies, and only a few relate to placebo-controlled RCTs (Hoffer *et al.*, 2008 & 2015; Nauman *et al.*, 2018) which constitute the test-bed of full acceptance for any therapeutic drug.

Nauman *et al.* (2018) in their review discuss several cases of IVC in clinical trials that have evaluated it as a single agent or in combination with arsenic trioxide or non-redox cycling agents. The median sample size of these studies was 17 (range, 3–65) and the IV dose of ascorbate ranged from 1 g daily to 1.5g/kg thrice weekly. They conclude that the evidence supporting the existence of an anticancer effect of IVC is mixed. Thus a randomised phase 11 trial (*NCT02516670*) involving 69 patients with prostate cancer yielded the inconclusive result on whether docetaxel works better when given with or without ascorbic acid in treating cancer. The only one reported randomised clinical trial on ovarian cancer patients showed a significant 8.5-week increase in progression-free survival and decreased adverse events of the intravenously administered ascorbate.

A few studies have evaluated the effectiveness of pharmacological IVC in the control of pancreatic cancer. Among these is an NIH registered phase 1 clinical trial (Welsh *et al.*, 2013) on patients with metastatic and node-positive pancreatic cancer that was subsequently advanced to a phase 11 study (*NCT01515046)*. The single patient in the phase 11 trial was administered the standard drug Gemcitabine (1000mg/m^2 weekly for three weeks of a cycle of 4 weeks) in combination with escalating IV ascorbate targeted to achieve a plasma level of 350mg/dL (biweekly infusions for four weeks). It was established that the concurrent administration of these drugs elicited progression-free survival for the five years of the trial, and was also well tolerated by the patient with no adverse effects.

A case study involving another patient afflicted with stage 4 pancreatic ductal adenocarcinoma and treated solely with intravenous pharmacological ascorbic acid (75–125g at

2–3 times per week) has also been recently reported. The patient survived nearly four years after diagnosis (Drisko *et al.*, 2018).

A phase I/IIa study to investigate pharmacokinetic interaction between IVC and gemcitabine by Polireddy *et al.* (2017) revealed that the ascorbate depleted cellular NAD^+ preferentially in cancer cells compared to healthy cells, leading to depletion of ATP and hence cell death, while substantially increasing α-tubulin acetylation in cancer cells which led to inhibition of motility and mitosis. Collagen was increased, and cancer cell epithelial-mesenchymal transition (EMT) was inhibited, accompanied by inhibition in metastasis.

Although the foregoing may suggest a mixed bag of outcome results for IVC in the treatment of a variety of cancers, some general conclusions can nevertheless be drawn whose relevance is particularly impacting on individuals who have sub-optimal amounts of the vitamin in their bloodstream. Among these are the following:

a) Oxidative stress induced by ROS and metabolic syndrome (via epigenetic changes in gene expression) are the leading causes of cancers, and the strong reducing properties of vitamin C, coupled with its critical function in several metabolic processes, gives the vitamin an exalted status as a first line antioxidant nutrient;
b) High dose vitamin C kills cancer cells by acting as a pro-drug, which delivers H_2O_2;
c) Chronic inflammation is the principal driver of malignant transformations, and IVC has been amply demonstrated in animal studies and in studies on human subjects by integrative and complementary cancer therapists to have a significant suppressive effect on the levels of pro-inflammatory cytokines;
d) Vitamin C manifests anti-angiogenic properties;
e) IVC inhibits the growth of many tumour cell lines in mice models, although less dramatically in human subjects;
f) Vitamin C may help alleviate some of the side effects of cancer treatment, but severe side effects caused by the vitamin itself when taken in high doses cannot be precluded;
g) IVC could interfere with some anti-cancer drugs, blunting their effectiveness from 30% to 70%, depending on the dose of vitamin C and the chemo drug; and
h) Encapsulated vitamin C may offer an alternative to patients who do not well tolerate IVC.

The need for well-planned, placebo-controlled RCTs involving a larger population mix and specific clusters of cancer type seems indicated before IVC's therapeutic benefit as an adjuvant in cytotoxic chemotherapy is accepted into mainstream medicine. In particular, cognisance needs to be taken by researchers that the response is likely to vary according to the initial vitamin C status of the subjects, and the presence or absence of other diseases or metabolic abnormalities.

A more in-depth study is also called for in developing a metabolic strategy for eradicating cancer stem cells (CSCs), which appear to be the root cause of tumour recurrence and therapeutic resistance, causing poor clinical outcome in advanced cancer patients. Given vitamin C's ubiquitous roles as an enzyme co-factor, the vitamin offers researchers a unique opportunity to use it as a combination therapy component in cancer treatment. This strategy has gained much attention in the cancer world since the recent report by De Francesco and co-workers (2017) that the combined use of the antibiotic Doxycycline and IVC offers a synthetic-lethal strategy for targeting metabolic flexibility in CSCs. The two-pronged attack targets cellular respiration, which is effectively reduced by the antibiotic through inhibition of mitochondrial protein translation, and glycolysis by the vitamin which causes ATP levels and adenylates energy changes in the cancer cells. This combination therapy was claimed to be a hundred times more effective than treatment with 2-deoxyglucose (2-DG) which serves to block the metabolism of glucose in cells.

B. Cardiovascular Disease (CVD) and Vitamin C

A possible role for vitamin C in promoting cardiac health has long been envisaged given the fact that it inhibits oxidation of LDL-protein, thereby reducing atherosclerosis. Oxidised LDL is a target for scavenger receptors, which

incorporate it into plaque (Salvayre *et al.*, 2016).

In as much as inflammation has a central role in the pathophysiology of CVD, and coronary artery atherosclerosis is characterised by lipid-driven chronic inflammation, this presages an essential role for vitamin C in suppressing the pro-inflammatory cytokines that are released in the initial cardiac insult. Implicit in this is the availability of vitamin C at adequate concentration levels in the plasma. Other factors that might favour expectations of a positive impact of vitamin C on cardiac risk reduction come from two early studies (Weber *et al.*, 1996, Woollard *et al.*, 2002) pointing to the vitamin's inhibition of adhesion of circulating monocytes to endothelial cells, a crucial step towards the formation of atheromas; its promotion of endothelial nitric oxide (d'Uscio *et al.*, 2003), a vasodilator that lowers blood pressure; and its conceivable role in preventing apoptosis of vascular smooth muscle cells (Siow *et al.*, 1993) which would help keep plaques more stable if atherosclerosis developed.

In the study by Woollard *et al.* (2002), 40 healthy adults were subject to vitamin C supplementation (250mg/day). Those who had low pre-supplementation levels of the vitamin had 30% greater monocyte adhesion than usual, which placed them at a higher risk for atherosclerosis. Impressively, after six weeks of supplementation, the rate of monocyte adhesion fell by 37%.

A randomized control study (Tahir *et al.*, 2005) involving 100 patients with mild-to-moderate aortic stenosis, broken up into control and two supplemented groups receiving vitamin C (1000mg/day) and both vitamins C (1000mg/day) and E (400IU/day), revealed that both supplemented groups experienced significant reductions in levels of several adhesion molecules - serum intracellular adhesion molecule (ICAM-1), E selectin, P selectin, vascular-cellular adhesion molecule (VCAM-1) - that would otherwise have served to promote inflammatory damage to the heart valves.

A reduced incidence of coronary heart disease by as much as 25% for subjects taking >700mg supplemental vitamin C/day was reported by Knekt *et al.* (2004) in a cohort study pooling nine prospective studies. Moser and Chun (2016) in their more recent exhaustive survey of observational cohort studies, however, note that many of the published findings are conflicting and less well substantiated on the vitamin's effect on CVD risk and mortality. They concluded that "*overall, current research suggests that vitamin C deficiency is associated with a higher risk of mortality from CVD and that the vitamin may slightly improve endothelial function and lipid profiles in some groups*".

A 10-year (EPIC-Norfolk) prospective cohort study involving 20,649 adult patients found that individuals with plasma vitamin C levels in the top quartile (25%) had a 42% lower risk of stroke compared to those in the lowest quartile; also, that serum levels of vitamin C were highly correlated with fruit and vegetable intake (Myint *et al.*, 2008). Stroke is the leading cause of permanent morbidity worldwide and reactive oxygen species, which are directly toxic to neurons and glia, have been seriously implicated in the development of acute ischemic stroke. While the antioxidant ascorbic acid does not penetrate the blood-brain barrier, its oxidised form, dehydroascorbic acid (DHA), enters the brain through facilitative transport. This has aroused considerable interest in DHA as a potent transportable form of vitamin C for achieving the desired supraphysiologic concentrations of the ascorbate for mediating potent cerebroprotection.

A recent study by Cisternas *et al.* (2014) on primary cultures of rat brain cortical neurons yielded the interesting result that DHA modulates the neuronal energy metabolism by facilitating the utilisation of glucose through the pentose phosphate pathway (PPP) for antioxidant purposes. In this study, DHA uptake by neurons triggered oxidative stress, as revealed by a decline in GSH. However, this was followed by a spontaneous restoration of the reduced glutathione status. This takes place at the expense of shifting glucose consumption from glycolysis to the PPP, a metabolic route that efficiently provides the reducing equivalents in the form of NADPH (H+) for GSH regeneration from its oxidised, GSSG form. Furthermore, their studies showed that DHA stimulated the uptake of lactate, a metabolic substrate that likely contributes to satisfying the high energy needs of neurons.

A more direct study of clinical interest was that carried out by Huang *et al.* (2001) on experimental stroke induced in mice. The animals were induced with reversible or permanent focal cerebral ischemia by intraluminal middle cerebral artery occlusion, and they were subjected to infusion treatment with vehicle solution (sodium

bicarbonate/sodium acetate buffer), AA, or DHA (40, 250, or 500mg/kg), either before or after ischemia. Given before ischemia, DHA caused dose-dependent increases in post-reperfusion cerebral blood flow, with reductions in neurological deficit and mortality. In reperfused cerebral ischemia, the mean infarct volume was reduced from 53% and 59% in vehicle- and AA-treated mice, respectively, to 15% in 250mg/kg DHA-treated mice (P <0.05). Similar significant reductions were also noted in non-reperfused cerebral ischemia. Delayed post-ischemic DHA administration after 15 min or 3 h also resulted in improved outcomes. The lack of increased intracerebral haemorrhage (ICH; assessed by spectrophotometric assay of haemoglobin in brain homogenates) after the delayed administration of DHA following ischemia is a particularly appealing result, given that the best current treatment for stroke victims within 3 hours of its onset entails the use of recombinant tissue plasminogen activator (rtPA) which carries with it the attendant risk of ICH and mortality.

The potentiality of DHA in the treatment of stroke is worthy of human clinical trials, especially among people with diabetes who are generally regarded as a high-risk group as oxidative stress and ROS formation are markedly increased by uncontrolled hyperglycemia. In this regard, it is interesting to note that an early 1982 study by Banerjee had established that compared with non-diabetic controls, diabetes patients and those with a hereditary predisposition to diabetes have low blood levels of AA but rather high DHA levels, irrespective of age, sex, history of diabetes, or treatment. This circumstance of DHA loading and AA depletion in the blood can combine to cause a significant decrease in GSH concentration, resulting in significant impairment of DHA reduction to ascorbate (Li *et al.*, 2001; Waly *et al.*, 2015). Evidence that glutathione synthesis is indeed diminished in patients with uncontrolled diabetes has been secured by Sekhar *et al.*, (2011). However, its restoration can be achieved by dietary supplementation with its precursors, namely cysteine and glycine, and orally stable acetyl glutathione or liposomal glutathione.

Overall, the evidence linking vitamin C levels to cardiovascular health, although stemming mainly from animal studies, is suggestive of a strong association. What is abundantly clear is that among humans the high-risk groups are the elderly, smokers, diabetics and hypertensives with lowered plasma vitamin C levels. A vital protective function of the vitamin that is fast gaining the attention of clinicians is its ability to increase the formation of collagen, elastin and other reinforcement molecules in the body. This improves the stability of the arteries and reduces the risk of plaque build-up.

The potentiality of using DHA in larger and well-designed clinical trials on diabetic stroke victims is gaining traction and promises to be a new frontier in epidemiological research on CVD.

C. Diabetes Mellitus Type 2 and Vitamin C

Given the fact that people with diabetes have an elevated risk of heart disease, and that diabetes is a primary pathological manifestation of oxidative stress in the body resulting from an imbalance between free radicals (ROS/RNS) and antioxidant, it was but a natural extension of epidemiological studies on the vitamin to investigate its effectiveness as an exogenous dietary antioxidant to neutralize ROS radicals that can disrupt the body's delicate homeostasis. Oxidative stress, however, is not the only contributory factor for the progression of diabetes; other well-defined factors include hyperglycaemia, inflammation, and insulin resistance.

A comparative study (Fadupin *et al.*, 2007) of serum ascorbic acid level in people with and without type 2 showed low basal vitamin C levels in patients with type 2 diabetes (T2DM) compared with control, although there was no difference in the intake of the vitamin itself by either group. The patients concerned in the study were administered an oral hypoglycaemic. The lowered level of the vitamin noted in the serum test of the patients was indicative of oxidative stress.

Alternative indications of diabetes pathogenesis can also come from an alteration in enzymatic systems, lipid peroxidation, and impaired glutathione metabolism (Asmat *et al.*, 2016). The effects of hyperglycaemia and hyperlipidaemia being additive in CVD, both lipid abnormalities and serum insulin levels were included for evaluation in a six-week study of 84 T2DM patients who randomly received either 500 mg or 1000 mg of vitamin C

(Afkhami-Ardekani, & Shojaoddiny-Ardekani 2007). A significant decrease in fasting blood sugar (FBS), triglyceride (TG), low-density lipoprotein (LDL), glycated haemoglobin (HbA1c), and serum insulin was seen in the group supplemented with 1000 mg vitamin C. The dose of 500 mg vitamin C, however, did not produce any significant change in any of the parameters studied.

A randomized double-blind, placebo-controlled, 12-week study by Ganesh *et al.* (2011) involving seventy type 2 diabetic patients has examined the effect of oral administration of vitamin C with metformin on FBS and post-meal blood glucose (PMBG), as well as on the levels of plasma ascorbic acid (PAA) and HbA1c in the patients.

Decreased PAA levels were found in patients with type 2 diabetes mellitus. This level was reversed significantly after treatment with vitamin C along with metformin compared to placebo with metformin. FBS, PMBG, and HbA1c levels showed significant improvement after 12 weeks of treatment with vitamin C at the oral dosage of 500 mg twice daily along with 500 mg metformin. These patients, who were on metformin, were not on any other medicine including other antidiabetic agents. It was concluded that oral supplementation of vitamin C with metformin reverses ascorbic acid levels, reduces FBS, PMBG, and improves HbA1c, thus favouring the use of both the drugs in combination in the treatment of type 2 DM to maintain reasonable glycaemic control.

Increased urinary albumin excretion is a marker of renal and cardiovascular risk in patients with type 2 diabetes. In a randomized, double-blind, placebo-controlled clinical trial, 69 type 2 diabetic patients were tested at the beginning and end of a 3 month period following supplementation of vitamins C (200mg/day) and E (100IU/day) and also of both these vitamins in combination with magnesium (200mg/day) and zinc (30mg/day) for improvements on their glomerular and tubular dysfunctions. Only urinary albumin excretion and not N-acetyl-beta-d-glucosaminidase activity declined over this period, indicating improvement only to the renal glomerular function (Favid *et al.*, 2017).

A systematic review and meta-analysis of RCTs testing the effect of vitamin C administration on glucose, HbA1c and insulin concentrations in adults (over 900 participants) has revealed that, while overall vitamin C did not modify any of these concentrations, there were significant differences in subgroup analyses (Ashor *et al.*, 2017). Thus, significant reductions in glucose concentrations were noted in T2DM patients and interventions exceeding 30 days. The vitamin's effect on fasting insulin concentration was also much higher than that observed for the postprandial case. In general, the results confirm previous observations that reduction in glucose concentrations was higher in patients with diabetes, older individuals and with more prolonged supplementation of vitamin C.

Lower vitamin C levels in diabetic patients have been seen as a consequence of diabetes itself and not due to the inadequate dietary intake of the vitamin (Sinclair *et al.*, 1994). The explanation for this possibly resides in the fact that in T2DM, hyperglycaemia inhibits the uptake of DHA by the red blood cells (Stankova *et al.*, 1984). For its transport into the cells, DHA requires the GLUT1 and GLUT 3 glucose transporters and thus is competitively disadvantaged when the glucose load is high. This effect may be overcome by a large intake of vitamin C. The consequent higher turnover of vitamin C in diabetes may hence underlie the need for higher dietary vitamin C requirements in T2DM patients.

Diabetes is characterised by impaired glucose utilisation by insulin. The insulin resistance also promotes endothelial dysfunction and leads to the development of metabolic syndrome which constitutes an introduction to cardiovascular disease.

The intimate linkage between endothelial and insulin signalling pathways, and hence between endothelial function and insulin metabolism, is well grounded in mechanistic details and is relevant to our understanding of pathological disorders such as hypertension, obesity and diabetes (Janus *et al.*, 2016). These authors note that involvement of insulin resistance and endothelial dysfunction in pathological disorders contribute to impairment in the NO-dependent vasodilatation, cellular glucose uptake, enhancement in oxidative stress, and inflammation, leading finally to atherosclerosis. RCTs in support of this cross relationship need to be widely undertaken, involving in particular diabetic patients, so that a potential therapy might emerge that can significantly

improve endothelial dysfunction and insulin resistance.

D. Metabolic Syndrome, Gout and Vitamin C

Metabolic syndrome or "adiposity" is a cluster of conditions - excess body fat around the waist, increased blood pressure, high blood sugar, and abnormal cholesterol or triglyceride levels- that occur together, increasing the risk of heart disease, stroke, fatty liver disease and type 2 diabetes. Subjects with metabolic syndrome suffer from an increased level of oxidative stress, and the chronic accumulation of body fat over time causes inflammation, insulin resistance and hyperglycaemia. Metabolic syndrome is a result of low circulating vitamin C concentrations that, according to Traber *et al.* (2018), may be traced to gut inflammation and impaired gut barrier function arising from excess dietary energy consumption. The authors propose that gut barrier dysfunction (leaky gut) leads to endotoxemia as a result of increased absorption of bacteria and gut-derived endotoxins, disrupts vitamin C absorption and consequently also vitamin E trafficking through a mechanism involving the gut-liver axis. This cycle of events that causes antioxidant depletion further increases chronic inflammation and oxidative damage.

A recently reported Korean study (Kim & Choi 2016) involving data analysis on 20,000 adults aged 20 years or older, chosen from the Korea National Health and Nutrition Examination Survey 2008–2012, has concluded that a combination of physical activity and dietary intake of vitamin C is what best provides for a lower risk of metabolic syndrome.

A more definitive study, albeit involving only a small cohort of subjects was that carried out by Mikirova's group (Mikirova & Scimeca 2016; Mikirova 2017) at the Riordan Clinic where they analysed the effect of IVC on mRNA levels of several genes involved in inflammation and stress response in subjects with metabolic syndrome. The gene expression modulation in peripheral blood mononuclear cells (PBMCs) from 20 overweight or obese subjects was determined. Participants were infused twice with 15,000mg ascorbic acid (AA), with a one-day interval between treatments. The following results were obtained: mRNA levels of Interleukin 4 (IL-4) and Interleukin 6 (IL-6) correlated with inflammation, as indicated by CRP levels; IVC resulted in a significant increase in blood AA and DHA concentrations; analysis of mRNA levels on PBMC before and after IVC showed down-regulation of genes coding for Interleukin 8 (IL-8) and up-regulation of Nuclear factor erythroid-derived 2 (NRf2), IL-4, Interleukin 10 (IL-10), Tumour necrosis factor alpha (TNF-α), and Interferon gamma (IFN-γ).

The transcription factor Nrf2 is the master regulator of the cellular redox homeostasis. It regulates the expression of several enzymes that synthesise antioxidants and detoxifying molecules and also enhances the expression of genes involved in cell energy production and maintenance (Holmström *et al.*, 2016). Nrf2 signalling is essential for detoxification of reactive metabolites and reactive oxygen species. This enabling activation of Nrf2 by IVC treatment is a significant finding as the vitamin can protect against age-related degenerative diseases and cancer.

Hyperuricemia along with hypertension and obesity are established risk factors for gout. Vitamin C has again been the focus of some attention by researchers seeking strategies for the risk reduction of these health problems.

A standout prospective study on the association of vitamin C with the risk of gout (the most common inflammatory arthritis in adult males) is that reported by Choi *et al.* in 2009, which followed several earlier metabolic studies attesting to the vitamin's effect in significantly reducing serum uric acid levels. This was thought to be due to greater removal of the urate through the kidneys. The prospective study was over 20 years with over 40,000 male participants with no history of gout at baseline. They documented some 1,300 confirmed incident cases of gout over this period; the vitamin C doses orally administered ranged from 250mg/day to 1500mg/day. For every 500mg increase in vitamin C intake, the risk for gout fell by 17%. The risk dropped by 45% when study participants took more than 1,500mg/day of vitamin C as a supplement. However, it is to be cautioned that rapid changes in uric acid levels can trigger a gout attack on people susceptible to gout. Such rapid changes may conceivably be brought on by drugs such as probenecid and sulfinpyrazone taken in conjunction with mega doses of vitamin C.

A meta-analysis of over two thousand RCTs bearing on the

effect of oral vitamin C supplementation on serum uric acid (SUA) levels on subjects, excluding children and patients on dialysis, has revealed that vitamin C significantly lowered SUA, but indicated the need for future trials to answer the questions on whether vitamin C supplementation can reduce hyperuricemia or prevent incident and recurrent gout (Juraschek *et al.,* 2011). A complicating factor here is that most people with hyperuricemia do not get gout.

The current consensus view is that though vitamin C may reduce the risk of developing gout, modest intake of the vitamin does not significantly lower uric acid levels in patients with established gout (Stamp *et al.*, 2013), and that the amount of vitamin C that would be ideally effective for gout without causing some of the harmful side effects of excessive vitamin C (such as kidney stones) still needs to be ascertained clinically.

E. Alzheimer's Disease and Vitamin C

Alzheimer's disease (AD) has presently become a widespread health problem, and researchers continue to look for interventions that could delay the onset of this chronic neurodegenerative disease.

The amyloid cascade hypothesis is considered the primary event of AD pathogenesis. It proposes that the deposition of β-amyloid (Aβ) is the first pathological event in AD leading to the formation of senile plaques (SPs) and then to neurofibrillary tangles (NFTs), neuronal cell death, and ultimately dementia (Reitz 2012). The ϵ4 allele of the apolipoprotein E (APOE) gene, which was identified as the first susceptibility gene for late-onset AD, is considered the major genetic risk factor for the disease (Slooter *et al.*, 1998), although reported risks vary tremendously among studies.

Observational studies point to low plasma vitamin C concentrations in AD patients than healthy individuals (Harrison *et al.*, 2014). Evidence that vitamin C plays a significant neuroprotective role in AD comes from mouse model studies, although evidence in humans yielded equivocal results. The neuroprotection arises not just from the vitamin's well-known scavenging activity against ROS, but also from its suppression of pro-inflammatory genes, mitigation of neuroinflammation, the chelation of iron, copper, and zinc, and the suppression of amyloid-beta peptide (Aβ) fibrillogenesis (Yao *et al.*, 2004; Choudhry *et al.*, 2012).

Several studies have examined the effectiveness of vitamin C if taken alone or in combination with vitamin E, beta-carotene or alpha-lipoic acid in combatting the onset of Alzheimer's disease. It is widely observed that people who develop AD usually experience a period of progressive cognitive decline before their AD diagnoses and that its occurrence is intermediate between healthy ageing and AD (Basambombo *et al.*, 2017).

For patients with Alzheimer's disease, early symptoms, such as mitochondrial dysfunction, often occur before some standard pathological features. Disorders of mitochondrial function lead to an increase in the level of reactive oxygen species (ROS), which in turn leads to the accumulation of beta-amyloid peptides, a process that accelerates the pathogenesis of Alzheimer's disease. Dixit *et al.* (2017) have pointed out that the presence of amyloid and unquenched ROS, in turn, contribute to mitochondrial dysfunction. They used mouse models to examine the effects of Alzheimer's-linked mutations and vitamin C deficiency on mitochondrial function.

Vitamin C deficiency led to diminished mitochondrial respiration and increased ROS, while mitochondria from the mouse model of Alzheimer's displayed increased respiration compared to wild type controls. The results suggested that both vitamin C deficiency and the presence of amyloid contribute to mitochondrial dysfunction but via differing pathways.

An independent study has also established that vitamin C deficiency leads to the development of amyloid plaques in the mouse brain (Cheng *et al.*, 2011).

A link between cognitive impairment in individuals and their low vitamin C plasma levels could not be firmly established using the Mini-Mental-State-Examination test on cognitively impaired individuals (Travica *et al.*, 2017). A more sensitive cognitive assessment test appears warranted, as also the need to have the measurements of the vitamin C concentration taken in the cerebrospinal fluid (CSF) rather than in the plasma as this might be more indicative of the vitamin C status of the brain. In the healthy brain, the content of vitamin C in CSF is highly concentrated

compared to plasma (2–4 times more, 150–400μmol/L) (Harrison *et al.*, 2009).

A double-blind placebo-controlled clinical trial involving 78 subjects with mild to moderate AD was undertaken by Galasko *et al.* (2012) to evaluate whether antioxidant supplements affected CSF biomarkers. The supplements – vitamin C (500mg/day), vitamin E (800IU/day) and alpha-lipoic acid (900mg/day) – were administered over 16 weeks, but they elicited no influence on CSF biomarkers related to amyloid or tau pathology, and thus exerted no clinical benefit to the patients.

Although there is little dispute that antioxidant vitamins such as vitamin C can reduce neuronal damage and death caused by oxidative stress (Monacelli *et al.*, 2017) and thus alter dementia's pathogenesis, current opinion suggests that avoiding vitamin C deficiency is likely to be more beneficial than taking supplements on top of a regular, healthy diet to combat AD.

The recent trend of research in AD has focussed on the epigenetic mechanisms of the disease and how nutrition can influence its course through the regulation of gene expression (Athanasopoulos *et al.*, 2016). Principal among the epigenetic mechanisms involved in AD are DNA methylation, histone modifications, and microRNAs, wherein ascorbate is known to play a critical role. More in-depth studies to unravel the many interrelated factors that underpin the pathogenesis of AD are called for, in particular, whether ascorbate deficiency is indeed the precipitating cause in the elderly, and if so, whether nutrition can help mitigate the progression of AD.

F. Viral Diseases and Vitamin C

Vitamin C is an immune-relevant micronutrient which is depleted in viral infections, and this deficiency seems to play a critical role in the pathogenesis of viral infections.

A large number of animal studies have indicated that vitamin C may alleviate or prevent infections caused not only by viruses but also by bacteria and protozoa. However, studies on human infections have not extended much beyond the common cold. Vitamin C administration does not decrease the average incidence of colds in the general population, but in physically active people there is a 50% less incidence of the infection. Also, the regular intake of vitamin C has been noted to reduce the duration of colds, suggesting a biological effect. However, the role of vitamin C in standard cold treatment remains to be clarified (Hemilä 2017).

A study on the potentiality of IVC in the treatment of Epstein-Barr viral infection has been reported (Mikirova & Hunninghake 2014), based on the data files of patients at the Riordan Clinic.

178 patients treated with IVC (7.5g to 50g infusions) showed elevated levels of EBV EA IgG (range 25 to 211AU) and 40 showed elevated levels of EBV VCA IgM (range 25 to 140AU). Most of these patients had a diagnosis of chronic fatigue syndrome (CFS), with the rest being diagnosed as having mononucleosis (i.e. an increase of white blood cells that have single nucleus- monocytes), fatigue, or EBV infection. Plasma levels of ascorbic acid and vitamin D were correlated with levels of antibodies to EBV. An inverse correlation was found between EBV VCA IgM and vitamin C in plasma in patients with mononucleosis and CFS meaning that patients with high levels of vitamin C tended to have lower levels of antigens in the acute stage of the disease.

Another study reported by Gonzales *et al.* (2014) concerns Chikungunya fever. This viral disease is produced by a single-stranded RNA Alphavirus from the *Togaviridae* genus and is transmitted principally by the mosquito, *Aedes aegypti*. The study was on an individual patient who was given IVC for two days which eliminated the symptoms.

High dose vitamin C treatment has also been shown to be effective against the avian virus H1N1 (Ely 2007) and the Zika virus (Gonzales *et al.*, 2016).

Many leading epidemiology researchers quoting clinical experience now advocate that intravenous vitamin C should be studied as a vital component of the treatment protocol for acute viral infections and their containment.

A multicentre prospective cohort study (Schencking *et al.*, 2012) involving 16 general practitioners and 67 participants with symptomatic herpes zoster, has examined the efficacy of IVC against shingles. The patients received vitamin C intravenously (7.5g/50ml) for approximately two weeks in addition to standard treatment. The assessment of pain and the dermatologic symptoms such as haemorrhagic lesions and the number of efflorescences were investigated in a

follow-up observation phase of up to 12 weeks. The results evidenced the vitamin's beneficial effects on herpes zoster-associated neuralgia, dermatologic findings and accompanying common complaints. Randomised, placebo-controlled clinical studies have been proposed to confirm the findings.

No significant clinical trials or *in vivo* human studies have been conducted to date to test the beneficial effects of high doses of vitamin C on AIDS patients, save for one early report by Cathcart (1984) who notes that massive doses of ascorbate (50-200g/day) can suppress the symptoms of the disease and can markedly reduce the tendency for secondary infections. However, a clinical remission occurs following the treatment which continues even with extended vitamin C infusions. This is despite continuing laboratory evidence of helper T-cell suppression. It was opined that there might be a complete or partial destruction of the helper T-cells during an initial infection that does not necessitate continuing toxicity from some source to maintain permanent or prolonged helper T-cell suppression. However, it is possible that ascorbate may prevent that destruction if used adequately during that prodromal period.

Some caution, however, seems indicated for HIV-positive people on antiretroviral in taking mega doses of vitamins, as this may raise alanine transaminase (ALT) levels which are indicative of liver damage (Isanaka *et al.,* 2012).

It is worth noting here that in vitro studies show that very high concentrations of vitamin C can prevent HIV from infecting new cells and prevent the activation and replication of HIV in dormant infected cells. Vitamin C also dramatically reduced the formation of syncytia, giant multi-nucleated cells arising from the fusion of virus-infected and uninfected T-cells. Syncytia tend to appear more frequently when CD4 cell counts are falling rapidly, and it has been inferred that their appearance may indicate an increased chance that AIDS-related illnesses will develop (Chowdhury *et al.*, 1992).

G. Other health disorders and Vitamin C

Vitamin C is one of the critical nutrients for good eye health. Its beneficial antioxidant effects in reducing the risk of cataract among older people (Ravindran *et al.*, 2011) have been well documented, but there is a lack of clear role for the vitamin on the primary prevention of age-related macular degeneration (AMD), as revealed by a recent Cochrane analysis (Evans & Lawrenson 2012).

Vitamin C, being a water-soluble antioxidant, protects body fluids and those fluids that surround the lens of the eye. Indeed, the function of the GABA receptors cells of the retina is critically regulated by vitamin C. Calero *et al.* (2011), in pointing this out, have conjectured that in as much as the retina is part of the central nervous system, the vitamin may have an important role as well in regulating the GABA –type receptors throughout the brain. The findings could have implications, according to these researchers, for other diseases like glaucoma and epilepsy. Both conditions are caused by the dysfunction of nerve cells in the retina and brain that become overexcited in part because GABA receptors may not be functioning correctly.

Vitamin C is a key component, along with thiamine (vitamin B1) and hydrocortisone, in a treatment cocktail for sepsis in a double-blind, placebo-controlled, adaptive randomised clinical trial that is currently underway in the United States involving some 2000 participants. The protocol calls for an intravenous infusion every six hours of vitamin C (1.5g), thiamine (100mg) and hydrocortisone (50mg) over four days or until ICU discharge. The clinical trial project – Vitamin C, Thiamine, And Steroids in Sepsis (VICTAS) Study 2018 - was inspired by the spectacular success achieved by Paul Marik and his team of doctors using this treatment cocktail of drugs for critically ill patients at Sentara Norfolk General Hospital in Virginia (Marik *et al.*, 2017).

IV. SIDE EFFECTS AND INTERACTIONS OF VITAMIN C

Vitamin C engenders no significant documented side effects or adverse interactions when used with other medications. However, large amounts of its use may not be advisable as it could, for example, (a) interfere with the effectiveness of warfarin (Coumadin, Jantoven) used to reduce formation of blood clots, and of protease inhibitors such as indinavir (Crixivan), a medication used to treat HIV and AIDS; (b) cause an increase of oxalate secretion in urine and thus risk kidney failure in people with kidney diseases; (c) cause red blood cells to break up in people with G6PD metabolic deficiency; and (d) cause an increase in iron absorption and thus worsen blood-iron disorders, including thalassemia and hemochromatosis.

(Ref: *http://lpi.oregonstate.edu/mic/vitamins/ vitamin-C*).

V. CONCLUSION

Vitamin C is a vital nutrient for health with its various therapeutic properties remaining under-exploited in primary stream medicine despite the success registered by practitioners of complementary and alternative medicine using pharmacological IVC. A shortage of high quality placebo-controlled clinical trials, and *in vivo* human studies remains a lacuna in concluding the vitamin as a safe adjuvant/ neoadjuvant or as a combination therapy component in treating some of humanity's major health problems. This needs to be addressed, and a balance needs to be struck between RCTs and control-based cohort observational studies.

Studies are also to be included on DHA as a potent transportable form of vitamin C for achieving supraphysiologic intracellular concentrations of the ascorbate where this may be desired. Where *in vivo* mouse-model studies are being engaged upon, it would be more prudent to study mice bearing a homozygous deletion of L-gulono-gamma-lactone oxidase gene; like humans, they require AA supplementation to avoid manifestations of vitamin C deficiency (Maeda *et al.*, 2000).

Orthomolecular medicine certainly has matured in recent decades and demands cognisance by the medical fraternity at large.

In many countries there is now mounting evidence, based on public health surveys, of widespread vitamin C deficiency in their populations, especially among low-income groups and smokers (Khan & Iqbal 2006, Mosdøl *et al.*, 2008). The designation "vitamin C" presupposes only small amounts are required. This is certainly not the case. An adequate intake of dietary and supplemental vitamin C (90-120mg/day) remains a pivotal personal health care need for both young and old to ward off a plethora of health problems.

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