S-4 심포지움초록

A Concentration-Function Basis for Ideal Vitamin C Intake

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ABSTRACT

Vitamin C is an essential nutrient involved in many functions. Humans are unable to synthesize vitamin C de novo, because they lack the last enzyme in the biosynthetic pathway. Previous Recommended Dietary Allowances (RDAs) for vitamin C were based on prevention of deficiency with a margin of safety. However preventing deficiency may not be equivalent to ideal nutrient intake. Recommendation should be based on vitamin function in relation to concentration. For this goal, data set of the relationship between wide-range of vitamin C dose and resulting concentrations in plasma and tissues and characterization of functional outcomes in relation to these concentrations should be acquired. This article reviews the current knowledge in these areas and suggest how this knowledge may contribute toward establishing dietary guideline for ideal vitamin C intake.

KEY WORDS: ascorbic acid, concentration, and function.

Vitamin C (ascorbic acid) is a six carbon lactone that is easily oxidized with the loss of one electron forming an intermediate radical, semidehydroascorbic acid or ascorbyl radical. As compared to other free radicals, ascorbyl radical is not a long-lived compound with a half-life of 10-5 seconds. Upon loss of a second electron, dehydroascorbic acid is formed. Dehydroascorbic acid can be reduced back to ascorbic acid via the same intermediate radical or hydrolyzed irreversibly to 2,3-diketogulonic acid. 2,3-diketogulonic acid is further metabolized into xylose, xylonate, lynxonate, and oxalate (Fig 1).1,2 The formation of oxalate has clinical significance because hyperoxaluria can result in oxalate kidney stones in some people.

Based on the redox potential of vitamin C and the stability of its intermediate free radical, vitamin C acts as an electron donor. As a specific electron donor for 8 enzymes, vitamin C plays an essential role in cells and tissues such as collagen hydroxylation, carnitine biosynthesis, and formation of the catecholamine norepinephrine.2 Also as a non-specific reducing agent (or antioxidant) for chemical reactions, vitamin C combines with a fairly reactive radical forming a much less reactive radical both inside and outside cells3, consequently influencing on lipid, protein and DNA. However most have been described in vitro and it is as yet uncertain that these effects have any biological relevance.

Vitamin C is synthesized de novo in the livers of most adult mammals, but humans and non-human primates, and guinea pigs cannot. These species lack the enzyme, gulonolactone oxidase, which catalyzes the last step in the endogenous synthesis of ascorbic acid from glucose. 4 Therefore humans depend entirely on dietary sources. Previous Recommended Dietary Allowances (RDAs) for vitamin C were based on prevention of deficiency with a margin of safety. 5, 6 However preventing deficiency may not be equivalent to providing optimal amount of vitamin C for human health. 7 If vitamin C toxicity were a problem, then preventing deficiency could be a practical goal for optimal ingestion. However vitamin C is well known as a non-toxic compound and dietary guideline for ideal intake of vitamin C should be established based on vitamin function in relation to concentration. To achieve this goal, following data sets must be obtained : (1) Vitamin concentration achieved in humans in relation to dose, across a wide dose range. How

different amounts of ingested vitamin C regulate circulating concentration and how circulating concentration regulates intracellular concentration should be considered. (2) Molecular and biochemical function in relation to vitamin concentration in cells. Findings must then be extended to humans.

The aim of the present article is to review the current state of knowledge in these areas and suggest how this knowledge may contribute toward establishing dietary guideline for ideal vitamin C intake.

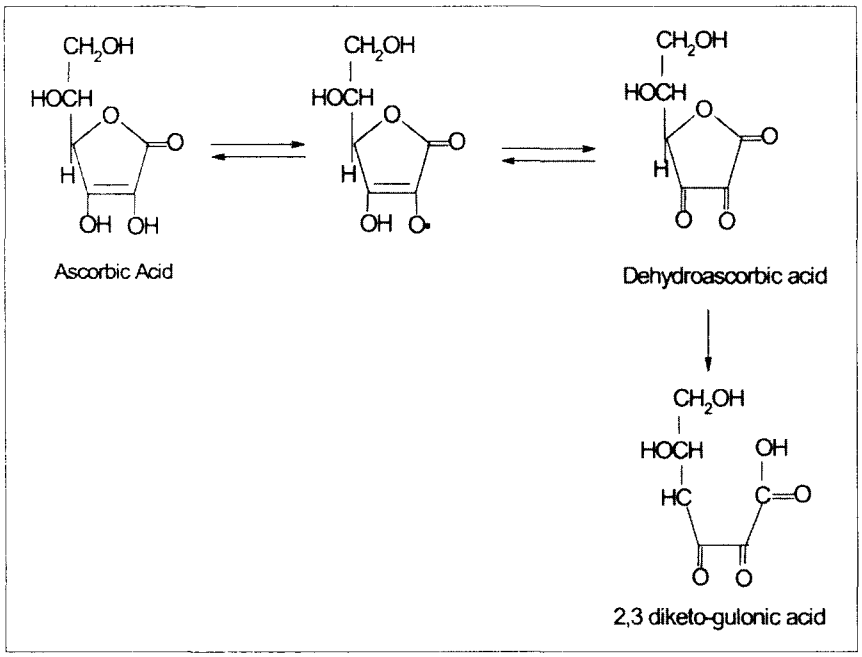

Fig. 1. Chemical structure of ascorbic acid and its oxidation products. Dehydroascorbic acid exists in more than one form, and only one is shown here for simplicity. Formation of 2,3-diketogulonic acid by hydrolytic ring rupture is probably irreversible (Modified from[21]).

VITAMIN CONCENTRATION ACHIEVED IN HUMANS IN RELATION TO DOSE

Although there are data available about vitamin C concentrations in humans8-18, most of them are limited and incomplete because of flaws in study design, execution, or analysis. Flaws included : use of vitamin C assays that were not specific or sensitive at low concentration ; narrow dose range of administered vitamin C ; use of a diet deficient in other vitamins and minerals ; use of radiolabelled vitamin C without verification of radiolabel metabolism in vivo ; lack of verification of steady-state for vitamin C dose ; and outpatient or uncertain dietary control of vitamin C ingestion.

Recently, new pharmacokinetic data about vitamin C were published, based on 7 men19 and 15 women20 who were in-patients for 5-6 months at the National Institutes of Health (NIH). Throughout hospitalization they consumed a diet containing less than 5mg of vitamin C daily. Deficiencies of other nutrients were prevented by supplementation. When plasma vitamin C concentrations achieved nadir $<$10uM, each 7 vitamin C repletion doses in water were administered twice daily either in fasting state or at least 90 minutes before meals until steady-state for the dose was achieved. Total daily doses administered in succession were 30, 60, 100, 200, 400, 1000 and 2500mg/day and the dosage were increased once a steady-state plasma concentration was achieved at each dose level. Vitamin C was measured by HPLC with coulometric electrochemical detection. Figure 2A shows fasting plasma vitamin C concentrations as a function of study day. Steady-state was attained when plasma vitamin C concentrations reached equilibrium for a given dose. An example of steady-state at the 60mg dose is shown in figure 2B.

Intake and Bioavailability

Dietary vitamin C ingested is absorbed in the gastrointestinal tract. Absorption is greatest in the proximal intestine21.

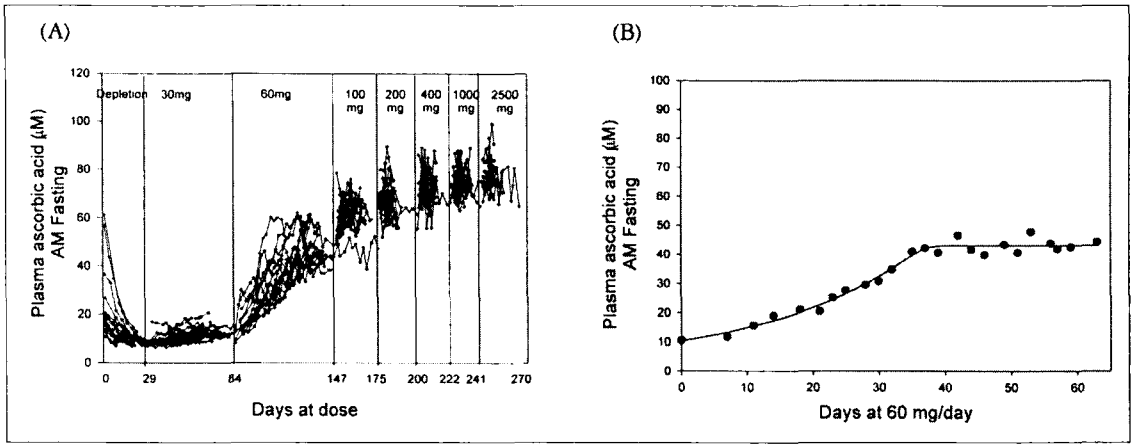

Fig. 2. Vitamin C plasma concentration as a function of days at dose in men. Each symbol represents a different subjects. (A) Vitamin C plasma concentrations were determined at least 2-3 times per week in all subjects. Subjects required different amounts of time to achieve steady-state at doses less than 100mg daily. Because some subjects remained at doses longer than other, gaps are displayed between doses. (B) Steady-state plasma vitamin C concentrations at 60 mg daily. (For details see[19])

It is unclear which form physically crosses intestinal membranes : ascorbic acid or dehydroascorbic acid or both. The data imply that both species are transported but by separate mechanisms : ascorbic acid by a Na+-dependent active transport system22 and dehydroascorbic acid absorption by a Na+-independent saturating process. 23

Bioavailability is a measure of efficiency of gastrointestinal tract absorption. Because of many difficulties, most investigators have estimated ascorbate bioavailability indirectly, such as comparing oral absorption to urine excretion24-27 or comparing absorption of one form of vitamin C (i.e., in foods) vs. another (i.e., in a supplement). 28-30

In the NIH studies, at each steady-state, true bioavailability was determined by comparing the increase in plasma after an oral dose with the increase in plasma after the same dose was given intravenously. Once an oral dose is given at steady-state, plasma values raise and then return to baseline. Then, the same dose is given intravenously. After an intravenous bolus, plasma values usually rise much faster because the gastrointestinal system is bypassed. Plasma values return to baseline again. Vitamin C bioavailability at steady-state for each dose was approximately 100% for 200mg, 73% for 500mg, and 49% for 1250mg (Fig 3). 100% bioavailability represents complete absorption.

Plasma Concentration and Cellular Distribution

In the NIH studies, steady-state plasma values were calculated for every subject at every dose and displayed as a function of dose for both men and women (Fig 4). There was a sigmoid relationship between dose and steady-state plasma concentration at doses below 400mg daily for both men and women. Especially over the range of 30-100mg daily, plasma vitamin C concentrations increased linearly with dose, with an approximate 5-fold increase. The curve for women was shifted to the left compared to that for men at doses of 30-100mg daily, meaning plasma concentrations for women at doses of 30-100mg daily were higher than for men. However differences disappeared at doses above 100mg daily. The first dose beyond the steep (linear) portion of the sigmoid curve for both sexes was 200mg daily. This dose produced a plasma concentration of approximately 70uM. At 400mg daily and higher, plasma concentrations were saturated at approximately 75-80uM.

Because vitamin C is water soluble, it is available to all tissues of the body by means of circulation. Uptake and accumulation of ascorbic acid in tissues is a function of plasma concentration, transport across cellular membranes, and mechanisms that maintain ascorbate intracellularly. Vitamin C content of human tissues varies over a wide range. Tissues with the greatest quantity of vitamin C include adrenal and pituitary glands, followed by liver, spleen, eye lens, pancreas, kidney, and brain. 31

In the NIH studies, tissue uptake of vitamin C was determined by measuring vitamin C concentrations in circulating

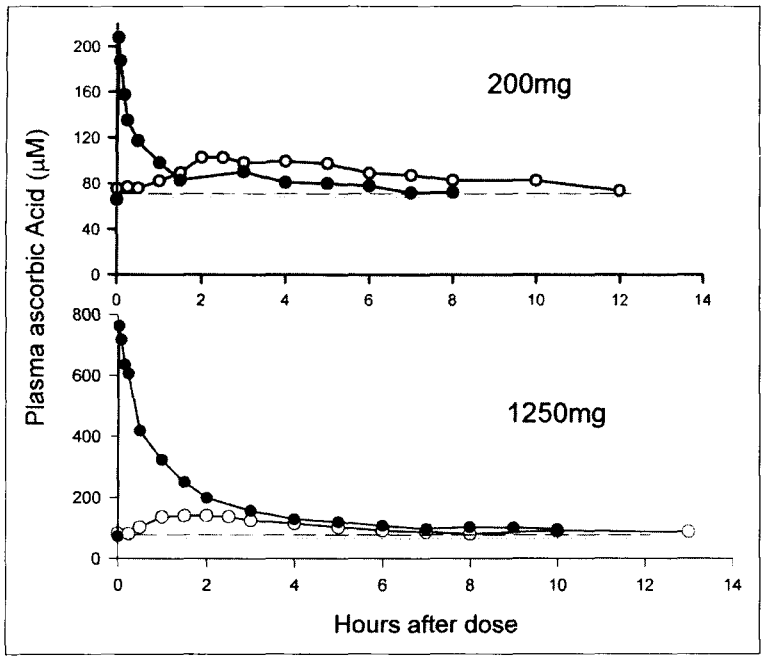

Fig. 3. Vitamin C bioavailability in plasma. The curve represent data from one subject. Vitamin C (200mg or 1250mg) was administered orally (○) at zero time (8 AM) and intravenously (●) after 24 hours each. Blood samples were obtained at the times indicated and plasma ascorbic acid was measured. Dashed lines indicate baselines. Bioavailability was the ratio of the area of the oral dose (are under the $curve_{po}$) divided by the area of the intravenous dose (area under the curveiv) (For details see[19]).

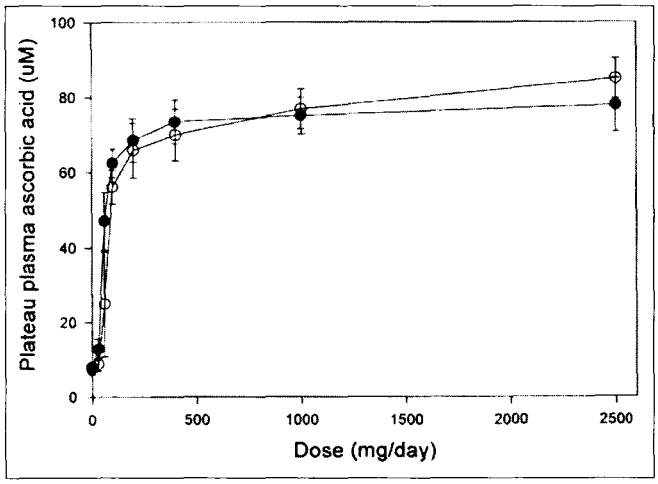

Fig. 4. Vitamin C plasma concentration as a function of daily dose in men and women. Steady-state concentrations were determined at 7 vitamin C daily doses from 30-2500 mg in men (○) and women (●). Values are means of plateau ascorbic acid at all doses. (For details see[19] & [20])

cells (neutrophils, monocytes, and lymphocytes) and platelets. Unlike other cells, circulating cells can be easily obtained. It has therefore been possible to examine the concentrations of vitamin C in these cells and the relationship between vitamin C intake and cell content in humans. The intracellular concentration of vitamin C in circulating cells and platelets saturated at the 100mg daily dose (Fig 5), which was less than the dose at which plasma vitamin C began to show saturation. At 100mg daily, intracellular vitamin C concentrations were 1-4mM, at least 14-fold higher than plasma. The corresponding plasma concentration at this dose was 55-60uM.

Urinary Excretion

Vitamin C is filtered at the glomerulus and is reabsorbed at the proximal tubule by an active transport process. 32,33 Maximal tubule reabsorption rates have been determined in men and women of different ages and found to be relatively constant between groups at about 1.5mg/100ml glomerular filtrate. 32 It is unknown whether vitamin C reabsorption can be regulated by other mechanisms, and it is not clear whether there is also active secretion of vitamin C into renal tubules.

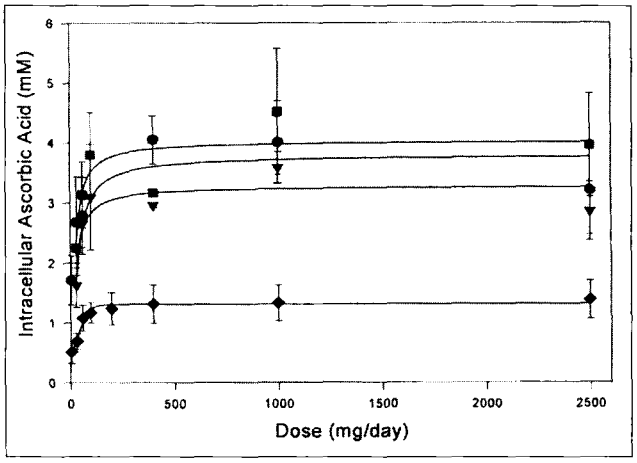

Fig. 5. Intracellular vitamin C concentrations in circulating cells as a function of daily dose in men. Neutroophils (◆), monocytes (▼), lymphocytes (●) and platelets (■) were isolated when steady state was achieved for each dose. (For details see[119])

In NIH studies, vitamin C urinary excretion was measured at steady-state for each dose (Fig 6). In men and women, the threshold dose for urinary excretion of vitamin C was between 60 and 100mg daily. With intravenous administration of 500mg and 1250mg, nearly the entire dose was excreted in urine. With oral administration of these doses, urine excretion was less than that of intravenous administration, probably because bioavailability was less at higher doses compared to lower ones.

Considered together, vitamin C concentration in plasma and tissues are tightly controlled as a function of dose in healthy men and women as a consequence of absorption, tissue accumulation and distribution, and renal excretion. Healthy humans apparently strive to reach plasma concentrations of 70-80uM, and once this concentration range is achieved it is not exceeded despite large increases in oral ingestion. Why tight control of vitamin C occurs in humans is not currently known.

However tight control of vitamin C concentrations could be bypassed for several hours when vitamin C was given intravenously. Independent of recommendations for physiological benefit, vitamin C given pharmacologically achieves far higher concentrations, and these perhaps might have therapeutic importance.

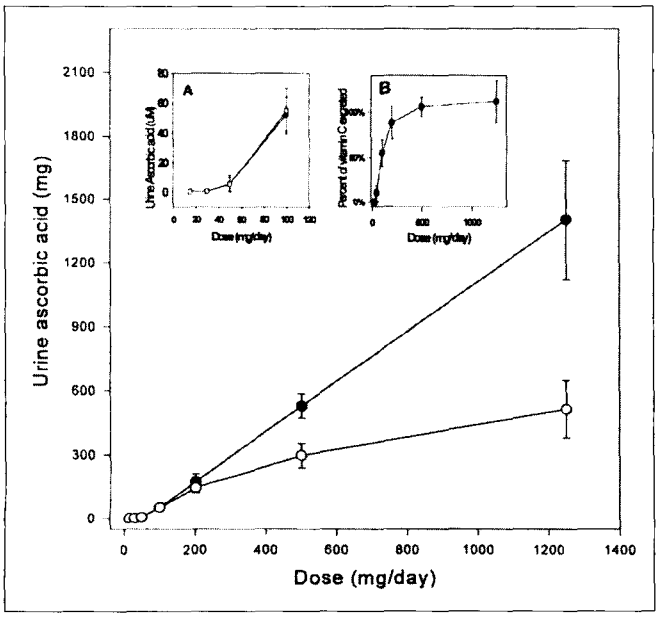

Fig. 6. Urinary vitamin C excretion as a function of single vitamin C doses at steady-state in men. Urine was collected over 24 hours during determination of vitamin C bioavailability for each dose. Vitamin C excretion was determined after administration of vitamin C given either orally (○) or intravenously (●). Inset A : Vitamin C excretion for single oral (○) or intravenous (●) doses of 15-100mg. Inset B : Fractional excretion after intravenous administration of single doses of vitamin C. (For details see[119])

To determine steady-state plasma concentrations and bioavailability, pure ascorbic acid was used. However, recommended dietary allowances provide guidelines for ingesting vitamin C in the diet, from foods. It is possible that other substances in foods rich in vitamin C could decrease absorption. For example, vitamin C is found in high amounts in many fruits and vegetables. Theses foods also contain many other compounds, with flavonoids as one example. A recent data34 showed that flavonoids inhibit the intestinal vitamin C transport when this transporter was expressed in expression systems (Fig 7A), when cells were transfected to overexpress the transporter (Fig 7B), and when bioavailability was determined in animals given vitamin C and flavonoids (Fig 7C). It is unknown whether flavonoids or other compounds in foods inhibit vitamin C absorption in humans. If such inhibition of absorption occurred, vitamin C dose concentration curves would be shifted to the right.

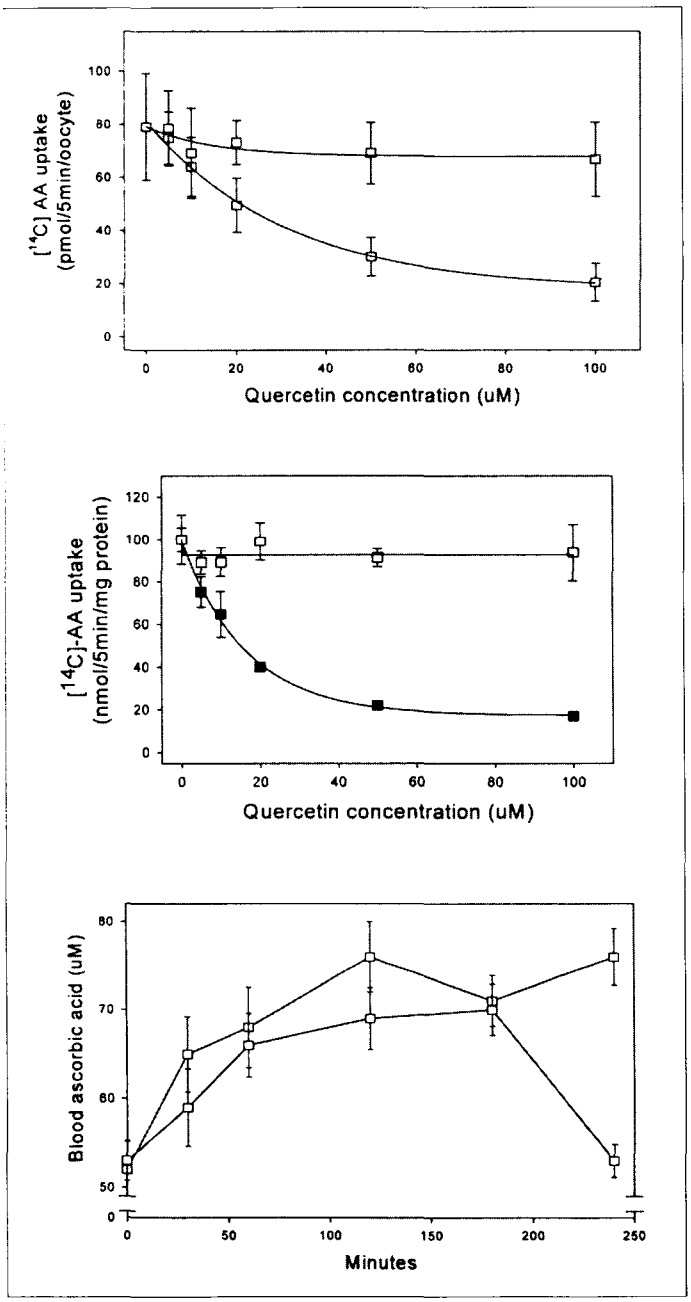

Fig. 7. *Flavonoid inhibition of ascorbic acid transport.* (A) In SVCT1(h) cRNA-injected X. *laevis* oocytes (B) In SVCT1(h) stably transfected Chinese Hamster Ovary (CHO) cells. (C) In Sprague-Dawley rats after overnight fasting received by gavage ascorbic acid (60mg/kg bw) with (■) or without (□) quercetin (15 mg/kg bw). (For details see[34])

MOLECULAR AND BIOCHEMICAL FUNCTION IN RELATION TO VITAMIN CONCENTRATION IN CELLS

There is no definitive data showing that vitamin C concentrations directly enhance molecular and biochemical function in human tissues, or that higher vitamin C concentrations confer benefit. Only indirect information is available regarding dose-function relationships. Based on the application of Michaelis-Menten reaction kinetics to vitamin C dependent reactions in situ, optimal intake for vitamin C can be determined. In simple terms, the kinetic measurements tell us the relationship between concentration and biochemical function for a variety of vitamin C dependent reactions. Because vitamin C saturation has no apparent adverse consequences, the amount of vitamin C ingested that provide the concentration resulting in maximum function (Vmax) but without toxicity would be the optimal intake.35

Transport

Vitamin C transport is a fundamental component for understanding in situ kinetics for different reactions. Cells acquire vitamin C through two distinct pathways. In one pathway, reduced vitamin C (ascorbic acid) is transported by Na+-dependent ascorbic acid transporter, SVCT1 and SVCT2.36 In a second pathway, oxidized vitamin C (dehydroascorbic acid) is transported by one of the facilitative glucose transporters, GLUT1, GLUT3 and GLUT4.37 Upon cell entry, dehydroascorbic acid is immediately reduced to ascorbic acid, which produces an effective gradient of dehydroascorbic acid across the membrane.38 The rate of dehydroascorbic acid uptake appears to be much greater than that of ascorbic acid for most tissues studied.39 However net transport is dependent on substrate availability.

Because dehydroascorbic acid is structurally similar to glucose, its entry has been proposed to be mediated by glucose transporters.40-43 Functional characterization of each glucose transporter isoforms GLUT1-5 for dehydroascorbic acid uptake were done in Xenopus laevis oocyte expression system. The apparent Km of DHA transport via GLUT 1 and GLUT3 was 1.1 ± 0.2 and 1.7 ± 0.3 mM, respectively (Fig 8).37

The cDNA sequences of human SVCT1 and SVCT2 were recently determined using an amplification strategy based upon the previously reported orthologs in rats, svct1 and svct2.36, 44-46 Sequence homology between the deduced human cDNAs is 65% for the amino acid sequence and 58% for the nucleotide sequence, scattered throughout the predicted coding region. Tissue expression patterns are different between the genes. SVCT1 is restricted to absorptive intestinal and renal tissues and the liver, whereas SVCT2 is ubiquitously expressed in most cell types.44,45 Chromosomal location of SVCT1 and SVCT2 was determined by fluorescent in situ hybridization analysis. SVCT1 mapped to the long arm of chromosome 544 and SVCT2 mapped to the short arm of chromosome 20.47

The Km for SVCT1 is approximately 10-fold higher than that for SVCT2 (Fig 9).36 Explanations of these observations might be different tissue distributions of the two transporters and available vitamin C concentrations in the different tissues. Based on their locations of SVCT1 in the small intestine, kidney and liver, the primary role of SVCT1 may be in intestinal absorption and renal tubular reabsorption of vitamin C. After ingestion of 100mg of vitamin C, an amount readily obtained from a meal containing fruits and vegetables, it is possible that intraluminal intestinal concentrations of vitamin C could be 200uM. Although the renal tubule concentration of vitamin C is unknown, it may be higher than the vitamin C plasma concentration due to reabsorption of sodium and water in the tubule before the site of vitamin C reabsorption. For these reasons, it can be predicted that the Km of the vitamin C transporter in intestine and kidney will be higher than that of SVCT2. Indirect evidence supporting the validity of the Km calculation for SVCT1 is that bioavailability of vitamin C is nearly complete a doses of 200mg, when intraluminal intestinal vitamin C concentration could be as high as 0.5mM.

Once inside cells, vitamin C serves as an electron donor for enzymatic and chemical reactions.2,48 Because vitamin C is involved in many distinct metabolic processes, aberrant transport could have implications for a variety of disorders. 49 Recently, SVCT2 knock-out mice has been created. Cultured embryonic fibroblasts from homozygous mice had

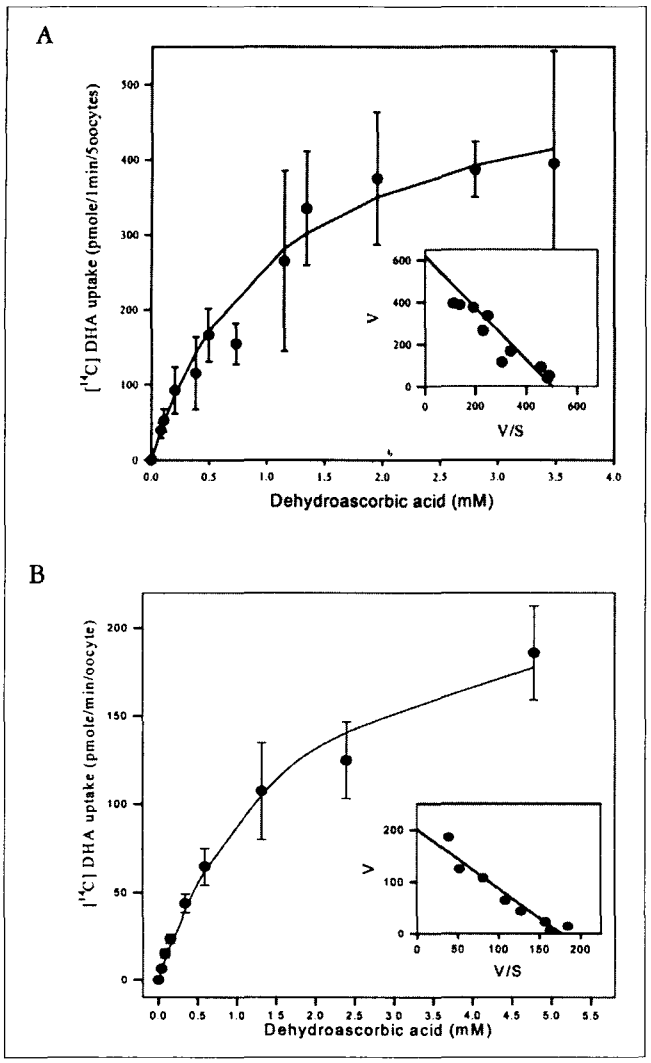

Fig. 8. Dehydroascorbic acid uptake by GLUT 1 and GLUT3. *X. laevis* oocytes expressing GLUT1 or GLUT3 were incubated with [^{14}C]-dehydroascorbic acid and quantified for radiolabel uptake. Internal reduction of dehydroascorbic acid to ascorbic acid was confirmed 100% by HPLC analysis at each concentration. Insets are Eadie-Hofstee transformation of the data. (A) GLUT1. K_m 1.1 mM, V_{max} 108 pmol/min/oocyte (B) GLUT3. K_m 1.7 mM, V_{max} 241 pmol/ min/oocyte (For details see[37])

less than 5% of normal vitamin C uptake. And homozygous mice died within a few minutes of birth with respiratory failure and intraparenchymal brain hemorrhage, suggesting SVCT2 is essential for transport of ascorbic acid into many tissues and across the placenta.50

Ascorbic Acid Recycling

Ascorbic acid recycling has been experimentally demonstrated in neutrophils.38 In resting cells, vitamin C is transported constitutively as ascorbic acid via SVCT2, and internal ascorbic acid can be maintained at mM concentrations. When neutrophils are activated by contact with bacteria, reactive oxygen species are produced leaked outside neutrophils and oxidize extracellular vitamin C to dehydroascorbic acid.38, 51 With increased availability of dehydroascorbic acid, this substrate is preferentially transported (10 fold faster than ascorbic acid) by glucose transporters, followed by immediate intracellular reduction (Fig 10). In this manner as much as 10 to 20-fold increases in intracellular ascorbic acid concentration can be rapidly achieved (Fig 11A). Ascorbic acid transport also occurs but at a much slower rate. Because ascorbate oxidized extracellularly is recycled intracellularly, the process is called ascorbate recycling. Vitamin C recycling is near maximal at an extracellular concentration of 75 umol/L.51

The results imply that dehydroascorbic acid formation and transport could occur during bacterial infection or inflammation.51 Rapid dehydroascorbic acid uptake and intracellular reduction may be a protective mechanism for cells.

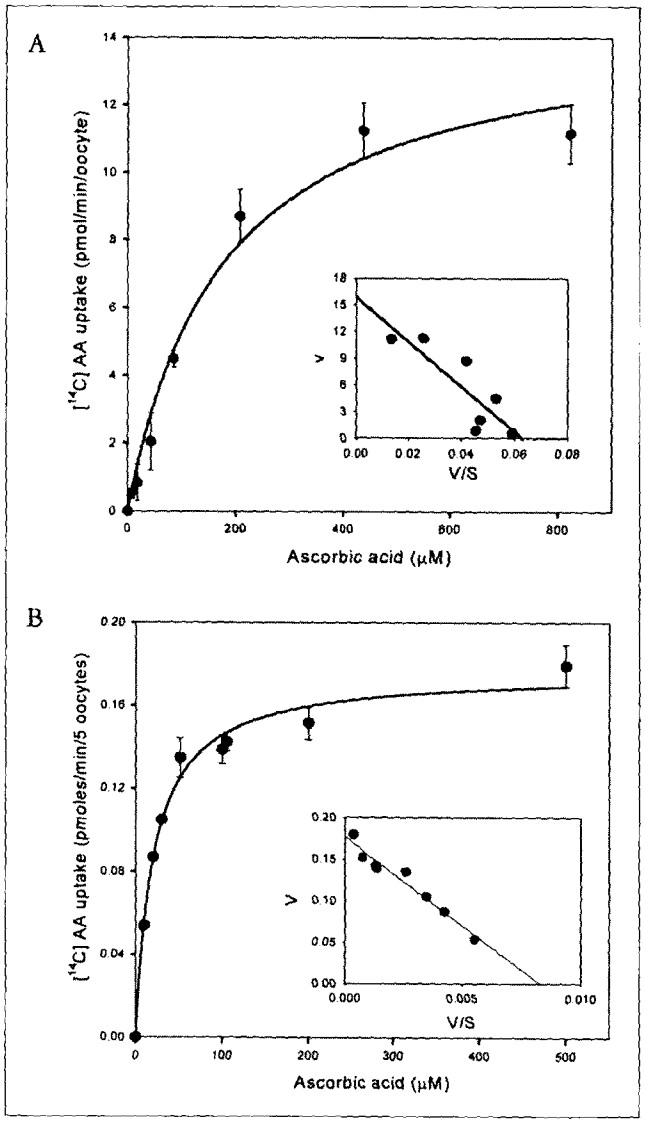

Fig. 9. Ascorbic acid uptake by SVCT1 and SVCT2. X. laevis oocytes expressing SVCT or SVCT2 were incubated with [^{14}C]-ascorbic acid and quantified for radiolabel uptake. Insets are Eadie-Hofstee transformation of the data. (A) SVCT1. K_m 237.3 uM (B) SVCT2. K_m of 22.2 uM (For details see[36])

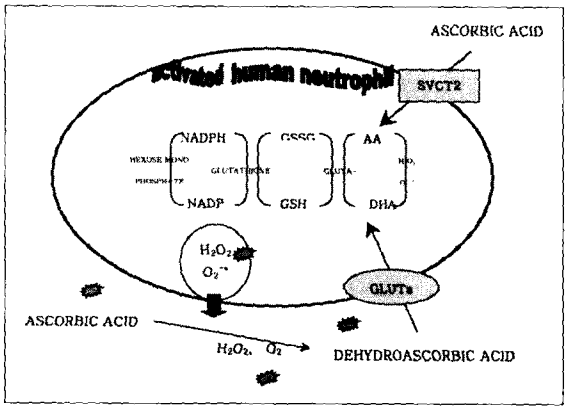

Fig. 10. A model of dehydroascorbic acid and ascorbic acid transport and recycling in human neutrophils. With bacterial activation, neutrophils secrete reactive oxygen species which oxidize extracellular ascorbic acid to dehydroascorbic acid, resulting in rapid uptake of dehydroascorbic acid and immediate intracellular reduction to ascorbic acid. As a result, as much as 10-fold higher internal concentrations of vitamin C are achieved. Dehydroascorbic acid entry may be mediated by GLUT1 and GLUT3 and ascorbic acid by SVCT2 in neutrophils. Abbreviations : AA, ascorbate ; DHA, dehydroascorbic acid ; GSH, reduced glutathione ; GSSG, oxidized glutathione ; (For details see[11&38])

The resulting sudden increase in intracellular ascorbic acid accumulation may blunt oxidative stress. Bacteria do not possess mechanisms to efficiently transport either ascorbic acid or dehydroascorbic acid, and do not accumulate ascorbic acid (Fig 11B). 51 Thus, recycling may confer a specific benefit to neutrophils during a bacterial challenge. If as-

corbic acid recycling can be demonstrated in vivo, the next challenges would be to determine the functional consequences of recycling and its regulation in vivo. Whether this mechanism is important in the function of immune cells and in protection against environmental oxidants has yet to be determined.

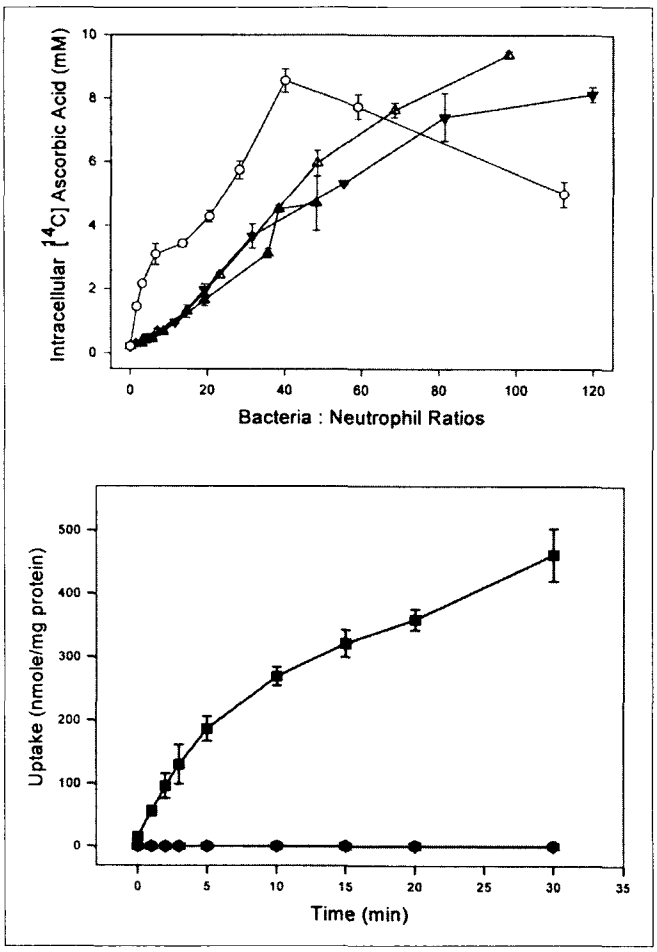

Fig. 11. Induction of ascorbic acid recycling in neutrophils. (A) Neutrophils were incubated with 100uM ascorbic acid with the indicated microorganism/neutrophiols (effector/target) ratios for the following microorganisms : *E. coli* (○), *M. catarrhlis* (□), *K. oxytoca* (▼), *C. albicans* (▲). Neutrophils incubated with ascorbate and no microorganisms are indicated by a big circle. (B) Bacteria (E. coli) were incubated with 400uM [^{14}C] ascorbic acid (■), [14C] dehydroascorbic acid (●), or [^{14}C] glucose (▲). Uptake was measured by scintillation spectrometry. (For details see[50])

CONCLUSION

The pharmacokinetic data from healthy young men and women define the concentration ranges of vitamin C in plasma and tissue at a wide range of vitamin C dose. Data describing similar dose-concentration relationships are needed in smokers, elderly subjects, and in patients with chronic diseases. Such information is essential for characterizing biochemical, functional, and/or clinical outcomes in relationship to vitamin C concentrations in healthy and ill people. To date, other than to treat deficiency, beneficial effects of vitamin C on clinical outcomes have not been conclusively demonstrated. Because functional and clinical outcomes are difficult measures in humans, but perhaps provide the most meaningful justification for ideal vitamin intake. Sound recommendations for nutrient intake are ideally made on the basis of clinical outcomes such as improvement in the quality of life, or reduction in morbidity or mortality.

In the absence of such information, surrogate markers and dose concentration relationships can be used to deduce ideal intake. Every effort should be made to use surrogate markers that are known to influence or determine clinical outcome.

National Cancer Institute of NIH recommended the ingestion of five servings of fruits and vegetables daily to pro-

tect against cancers of the GI and respiratory tracts, with potential benefit in preventing heart disease. Five servings of fruits and vegetables provide 210–280 mg of vitamin C daily. The available data suggest that ideal vitamin C intake is 200mg daily and vitamin C doses of 1g or more could have adverse consequences in some people. Taken together, for most adults the best advice is to eat five servings of fresh fruits and vegetables daily to maximize health, and to obtain vitamin C from these foods.

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