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# Ascorbic Acid Intake and Oxalate Synthesis

**John Knight**[1], **Kumudu Madduma-Liyanage**[1], **James A. Mobley**[2], **Dean G. Assimos**[1], and **Ross P. Holmes**[1]

[1]Department of Urology, University of Alabama at Birmingham, Birmingham AL USA 35249

[2]Department of Surgery, University of Alabama at Birmingham, Birmingham AL USA 35249

## Abstract

In humans approximately 60 mg of ascorbic acid (AA) breaks down in the body each day and has to be replaced by a dietary intake of 70 mg in females and 90 mg in males to maintain optimal health and AA homeostasis. The breakdown of AA is non-enzymatic and results in oxalate formation. The exact amount of oxalate formed has been difficult to ascertain primarily due to the limited availability of healthy human tissue for such research and the difficulty in measuring AA and its breakdown products. The breakdown of 60 mg of AA to oxalate could potentially result in the formation of up to 30 mg oxalate per day. This exceeds our estimates of the endogenous production of 10 – 25 mg oxalate per day, indicating that degradative pathways that do not form oxalate exist. In this review we examine what is known about the pathways of AA metabolism and how oxalate forms. We further identify how gaps in our knowledge may be filled to more precisely determine the contribution of AA breakdown to oxalate production in humans. The use of stable isotopes of AA to directly assess the conversion of vitamin to oxalate should help fill this void.

## INTRODUCTION

Despite over half a century of research on the relationship between ascorbic acid (AA; also known as vitamin C) intake and calcium oxalate stone disease the nature of this association is still blurred. The first report that AA could be converted to oxalate in humans was in 1958 by Hellman and Burns[1]. They reported that oxalate was the major product of AA excreted in urine. This report clearly triggered interest that AA consumption and the oxalate it could produce was involved in calcium oxalate stone formation. In the mid-1960's Atkins et al and Baker et al quantified the breakdown of ingested AA to oxalate using isotopes[2, 3]. They estimated AA breakdown contributed > 40% of the oxalate excreted in urine. Their analytical tools were limited by today's standards and it is unclear whether they took adequate steps to limit the in vitro conversion of AA to oxalate during analysis. Subsequent research confirmed that breakdown of AA to oxalate could occur in urine and increased with time, temperature and pH, but also revealed that it could be partially prevented by EDTA and urine acidification[4, 5]. More recent research has indicated that with even low levels of dietary AA consumption, small increases in intake (> 281 mg/day vs < 105 mg/day) in male health

Corresponding Author: Ross P. Holmes, rholmes@uab.edu, Ph. 1-205-996-2291.

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professionals increased stone risk by 31%[6], and total AA intake increased stone risk 2 fold in a large Swedish population[7]. At the other extreme, individuals constantly consuming large oral doses of AA or receiving intravenous infusions, have been reported to develop oxalate nephropathy in several case reports[8–12]. In contrast, a short term intravenous infusion of up to 100 g AA over a 6 hr period has been used as a pilot cancer treatment without any reported short term adverse events[13]. A large increase in urinary oxalate excretion did accompany such infusions. In between these extremes, a significant number of individuals consume daily AA supplements between 1 and 10 g for extended periods of time as either a protective or therapeutic agent and have an increased urinary oxalate excretion[14]. We will review this data, identifying its limitations and weaknesses, and will describe the current view on the breakdown of AA to oxalate, the only mechanism that has been seriously considered as a cause for the oxalate nephropathy and oxalate stone disease associated with AA ingestion.

## FUNCTIONAL ROLE OF AA

Humans similar to other primates, guinea pigs, some fish and some bats require AA because in these species the activity of a key enzyme involved in AA synthesis, gulonolactone oxidase, is absent[15, 16]. The gene encoding this enzyme still exists in the human genome but contains several mutations that make it non-functional[16]. It is believed this loss of function occurred over 45 million years ago and that it was probably the result of some evolutionary pressure. Halliwell speculatively proposed that the loss of this enzyme and urate oxidase in humans were steps to lower hydrogen peroxide production[15]. Grano and De Tullio further suggested that the inability to synthesize AA could make it an oxidative stress sensor as it is required for the hydroxylation of the hypoxia inducible factor (HIF) to induce an hypoxic stress response[17].

The major functional role usually ascribed to AA is its role as a water soluble antioxidant. It has the ability to scavenge free radicals forming a more stable ascorbyl radical. Two of these radicals can react to form AA and dehydroascorbic acid (DHA), the oxidized form of AA[18, 19]. Due to the multiplicity of antioxidant defense mechanisms in the body, however, it is not clear that this important activity is compromised by AA deficiency alone. The hallmark symptoms of AA deficiency (scurvy) result from an abnormal collagen synthesis due to impaired proline and lysine hydroxylation. AA is required for a variety of biosynthetic pathways, particularly those involving hydroxylation and amidation reactions. These reactions, besides those involved in collagen biosynthesis, include the hydroxylation of dopamine to norepinephrine in chromaffin granules of adrenal medulla, the α-amidation of glycine in pituitary gland peptides, prolyl hydroxylation in HIF, and histone demethylation[18].

## DIETARY SOURCES OF AA

The daily intake of AA currently recommended by the Institute of Medicine in the U.S. is 75 mg/day for adult women and 90 mg/day for adult men. According to the NHANES survey in 2009 – 2010 the average AA intake for adult males was 96 mg/day and 87 mg/day for adult females. If the assumption is made that ~20 mg/day of AA is excreted undegraded and the

remainder is broken down in the body, the yield of oxalate from breakdown of AA could potentially be as high as 39 mg/day in males and 34 mg/day in females. This is clearly an overestimate as the average urinary oxalate excretion is ~30 mg/day[20]. Dietary oxalate and the metabolism of hydroxyproline, glycine and glyoxal also contribute to urinary oxalate excretion[20–22]. Dietary oxalate absorption on average may contribute 12 mg/day (the absorption of 8% of an average daily intake of 150 mg)[23, 24]. Our analyses of the contributions of hydroxyproline, glycine and phenylalanine degradation to urinary oxalate excretion[21] (unpublished observations) indicate that they contribute at most 25% (7.5 mg) to the oxalate excreted by normal individuals. These estimates indicate that AA must be metabolized by pathways other than those that result in oxalate formation and that its breakdown could lead to the formation of up to 10.5 mg oxalate/day.

Some of the major dietary sources of AA are shown in Table 1. Fruits and vegetables are significant sources. Some meats such as liver can also contribute substantially. The content may vary depending on the cultivar, growth and storage conditions, as well as the method of food preparation. For example, one third of the AA was lost on storage of spinach at 4°C for 2 weeks[25]. As the AA in food degrades, oxalate may very well be a product. Oxalate was a major breakdown product of AA added to drinking water that had become contaminated with copper after flowing through copper pipes[26]. AA could also potentially breakdown to oxalate in any food or beverage fortified with this vitamin. Perusal of the shelves at grocery stores reveals an ever increasing supply of juices, baby foods and snack bars that contain supplemental AA.

## ABSORPTION, CIRCULATION, TISSUE UPTAKE AND URINARY EXCRETION

The absorption of AA in the human gut has not been fully characterized. A sodium dependent AA transporter (SVTC1) encoded by the gene SLCA23A1 has been identified in the small intestine of mammals and appears to play a significant role in AA absorption[27]. However, knockout mice lacking SVTC1 have a normal plasma AA. This led Corpe et al to identify the glucose transporters, GLUT2 and GLUT8, as also contributing to AA absorption[28]. Interestingly, plasma AA levels in these mice responded much better to the gavage of the oxidized form of AA, dehydroascorbic acid (DHA), than they did to gavage with AA. One interpretation of these results is that normally DHA is produced from ingested AA in the gut and then absorbed. It is then converted back into AA after internalization within epithelial cells. Oral doses of AA up to 200 mg/day are well absorbed (70 – 90%) but above this dose absorption declines and at 1250 mg/day it is ~33%[29, 30]. Ingested AA begins to be observed in urine at doses above 50 mg/day[30]. Factors that may influence the oxidation of AA in the gut and its absorption are largely unknown. Whether non-absorbed AA can breakdown in the gut to oxalate is also unknown.

Assessments of the AA distribution in tissues have been limited. Studies using single oral doses of $^{13}C$- and $^{14}C$-isotopes have suggested that there are 2 major pools of AA in the body[31, 32]. Our estimates of the size of these pools and average 24 hr urinary excretions are shown in Fig. 1. The first pool consisting of plasma and the extracellular fluid compartment

contains 150 mg of AA. The second compartment presumably represents the AA within cells and is ~ 1 – 1.5 g. Muscle is the largest tissue store containing ~ two thirds of the tissue pool despite containing one of the lowest AA concentrations of all tissues[33]. The concentration in each tissue compartment may differ due to its complement of transporters and the unique AA metabolism that might occur. Given the long interval taken for scurvy to surface after consumption of an AA-deficient diet it is clear that tissue cells have a remarkable capacity to retain and recycle AA. This slow flux appears to be bi-directional with 3 – 5 weeks taken for individuals to reach a stable concentration when adjusting from one level of dietary AA to another[30, 34]. For comparative purposes, estimates of oxalate compartmentation are also provided in Fig. 1. These estimates are based on values obtained for human liver tissue[35], mouse kidney[36] and food table values for beef muscle (0.1 mg/100 g).

As discussed above in the gut, AA uptake in cells appears to result from the activities of GLUT transporters that have a higher affinity for DHA than AA, and specific AA transporters (SVTC). With the recommended daily intake of AA these transporters result in a plasma AA concentration of 50 – 60 μM and a DHA concentration of ~1μM. Efflux of AA from cells can occur by several mechanisms with the main contributors believed to be volume-sensitive channels and $Ca^{2+}$ -dependent channels[37].

Elimination of AA from the body is believed to be almost entirely through the kidney[38]. Proximal tubules contain SVTC1 which appears to play a role in the reabsorption of filtered AA in conjunction with GLUT transporters[27]. With oral doses of AA of < 60 mg/day, < 0.4 mg/day is excreted[30] although 1500 mg/day is filtered. When the oral dose reaches 100 mg/day, ~25 mg/day is excreted[30]. The maximal plasma AA level reached with oral doses is ~0.25 mM[39]. The combined data on absorption and excretion indicate with oral doses over 1 g/day, a significant portion is not absorbed and the bulk of that which is absorbed is excreted unchanged in urine[30, 38]. AA may have clinical utility in treating cancer and infections but to be effective it must be given intravenously to raise the plasma AA to therapeutic levels[39]. Levels of 25 – 50 mM have been obtained with this approach[13, 40]. Recent research suggests it may act by inhibiting glycolysis through its effect on glyceraldehyde 3-phosphate dehydrogenase[41].

## METABOLISM OF AA

Circulating AA can be taken up and concentrated in cells and tissues where it can act as an antioxidant resulting in the formation of the ascorbyl radical[18]. The loss of a further electron results in the formation of DHA. Two ascorbyl radicals can readily dismute to AA and DHA. DHA can also be converted back to AA through an interaction with reduced glutathione. Glutaredoxin and possibly other enzymes have been reported to play a role in this reduction[19, 42]. The formation and removal of DHA is potentially important for oxalate formation from AA as a fraction of the DHA irreversibly forms the open chain diketogulonic acid (DKG), an unstable molecule that can break down to oxalate[43]. The conditions that promote or limit the breakdown of both DHA and DKG have not been well characterized. This is a major limitation in determining the contribution of AA metabolism to oxalate synthesis. The only work published is that from over half a century ago using outdated

methodology coupled with a lack of awareness of the susceptibility of AA to breakdown at alkaline pH[1–3]. Studies on the breakdown of DHA and DKG in phosphate buffer by Simpson et al have indicated that DKG rapidly splits into L-erythrulose and oxalate[43]. In lens homogenates it was reported that erythrulose can be converted to L-threitol by sorbitol dehydrogenase[44]. Possible pathways that may be involved in AA breakdown are shown in Fig. 2.

The analysis of AA and its degradation products has been an analytical challenge for decades. Poor stability in solution and the lack of suitable detection techniques have hampered achieving high selectivity and sensitivity in analyses. Classical analytical techniques such as titrimetric[45], spectrophotometric[46] and enzymatic methods[47] employing the AA/DHA redox chemistry have been used in the past but they lack specificity and are prone to the interferences inherently present in biological samples.

High performance liquid chromatography (HPLC) has become the preferred method of AA analysis in the past two decades. UV-Vis Absorption detection has been extensively used but coulometric electrochemical detection (ECD) has proven to have much higher specificity and sensitivity and is now widely used for the determination of AA in biological samples[48]. An electrochemical detector only visualizes analytes that are active at the electrode potential applied during the detection process and therefore is less susceptible to interferences present in the sample matrix. Even though ECD is believed to be less prone to interferences, during our recent studies on plasma AA content, we have observed that in plasma uric acid is also visualized at the same potential and elutes close to AA. It is essential that chromatographic conditions be optimized to obtain the separation required.

Considering the challenges in detecting and accurately measuring AA and its degradation products in biological specimens, mass spectrometry (MS) appears to offer superior detection and specification[49]. For this reason, presently an MS detector coupled to an HPLC system (LC-MS) is the most commonly applied approach for the analysis of AA derived from biological specimens, where analytes are separated on a HPLC column and then identified initially by their mass:charge (m/z) ratio. The main requirement for MS detection is the ability for the analyte(s) of interest to form positive or negative ions in the gaseous state, in which case AA can form both, and the parent mass can be measured with high accuracy depending on the MS instrument of choice. In the case of AA specifically, this analyte is generally quantified by LCMS using electro-spray ionization (ESI) in negative ion mode, as this provides optimal sensitivity[49–51]. With the current availability of more sophisticated MS instruments, high mass accuracy combined with tandem mass spectrometry MS(MS2) has become very common. The latest MS(MS2) instruments offer very high specificity and quantification for analyte(s) in complex matrices due to the ability to selectively monitor the parent analyte fragment ions (otherwise known as daughter ions). In this regard, Fenoll et al. reported a method to simultaneously determine AA and DHA in fruit extracts using LCMS(MS2) operated in negative ion mode while monitoring the resultant daughter ions in what is referred to as selected reaction monitoring (SRM)[52]. This is most likely the method of choice to measure DHA in plasma and tissues as current methods rely on DHA being converted to AA in samples and measuring the subsequent change in total AA[48]. This approach is inaccurate due to the large differential between AA

and DHA concentrations. GCMS or GCMS(MS2) is another mass spectrometric technique capable of analyzing AA and degradation products. Derivatization will be required to make these molecules volatile. Bluck et al highlighted the promise of this approach with a constant intravenous infusion of $^{13}C_1$-AA for 24 hrs in human subjects[31]. The slow decline in isotope enrichment in the extracellular pool was consistent with the remarkable ability of the body to retain AA.

With the use of LCMS(MS2) technologies, Nemet et al. recently identified five degradation products of AA, including DHA, DKG, 3-deoxythreosone, xylosone and threosone, in the human lens by derivatization with o-phenylenediamine and analysis in positive ion mode[44]. This group was able to identify 3-deoxythreosone, which originates from the decomposition of erythrulose as a major non-oxidative in vivo degradation product of AA, and this observation is consistent with those of Simpson et al[43]. Nemet et al also concluded that under oxidative conditions erythrulose can be converted to threosone. Wang et al. reported an LCMS(MS)2 method for the determination of L-threonate present in human plasma and urine using the ion transition from the parent ion of 134.5 *m/z* to the fragmented daughter ion of 74.7 *m/z* (134.5 → 74.7) as measured in negative ion mode[53]. They also observed that threonate is stable for 24 hr at room temperature and up to 3 months at −30°C. This observation is consistent with threonate being one of the major end-products of AA degradation. In a similar fashion, Szultka et al. more recently reported the identification of DHA, DKG, threonate and oxalic acid in aqueous AA solutions at different pH values and $H_2O_2$ concentrations, as measured by LCMS(MS2) in negative ion mode[51]. They proposed an AA degradation pathway similar to previous reports and observed products associated with hydration of DHA. Deutsch et al. also reported a mechanism for the hydrated hemiketal form of DHA[54]. These reports coincide with our recent observations using NMR (nuclear magnetic resonance) spectroscopy of aqueous DHA solutions (Fig. 3), which revealed the presence of two distinct forms of DHA (also depicted in the NMR databases: http://www.hmdb.ca/spectra/nmr_one_d/1674). A better understanding of the chemistry of DHA is vital, as this molecule is believed to give rise to the very unstable DKG, which spontaneously breaks down to oxalate. A clearer picture of the other degradation products formed from DKG is also required to characterize what could be several different degradative processes. We propose that by infusing healthy subjects with a stable $^{13}C_6$ isotope of AA, as we have previously performed with $^{13}C_2$-glycine infusion[21], coupled with an optimized LCMS(MS2) technique, we will be able to more precisely determine the contribution of AA degradation to endogenous oxalate production.

## EPIDEMIOLOGICAL EVIDENCE THAT AA CONTRIBUTES TO KIDNEY STONE DISEASE

The association between AA intake and kidney stone formation was first reported by Taylor et al in a prospective study of ~ 50,000 male health professionals[6]. Men with an intake >218 mg/day had a 31% higher risk of forming stones than those consuming < 105 mg/day (P = 0.01). Supplemental AA also increased risk with an intake > 1000 mg/day associated with a 16% increase over those taking no supplemental AA. In a similarly sized study in Sweden, supplemental AA intake was associated with a near 2-fold increase in stone risk[7]. This risk

appeared to be associated with the number of supplemental tablets taken each day, but the dose relationship was not accurately quantified. These findings suggest that AA intake could be a significant risk factor for the development of kidney stones and indicate that studies to determine the effects of both dietary and supplemental AA on stone risk factors, particularly urinary oxalate excretion, in normal subjects and stone formers consuming controlled diets are clearly warranted.

## ASCORBIC ACID INGESTION AND URINARY OXALATE EXCRETION

Because oxalate is formed from the breakdown of AA, an increased formation of AA resulting in an increased urinary excretion of oxalate is the only mechanism that has been considered for the stone risk associated with AA ingestion. It remains possible that some other less obvious mechanism is involved. Numerous studies have examined the association between supplemental AA ingestion and stone risk parameters and have reported an increased urinary oxalate excretion. The validity of many of these studies can be questioned, however, due to the possible formation of oxalate in urine samples either during storage or during analysis. Levine et al showed that when 400 mg of supplemental AA was given to normal subjects on a controlled, AA-deplete diet, urinary oxalate excretion began to increase[30]. As dietary oxalate was not controlled in these studies, it is possible that this limited their ability to detect the effects of levels of AA below 400 mg/day on urinary oxalate excretion. Traxer et al observed a 21.8% increase in urinary oxalate excretion in normal subjects receiving 2 g of AA on a controlled diet and a 34.4% increase in stone formers on the same diet[55]. Baxmann et al reported a 56% increase in urinary oxalate excretion in normal subjects consuming 1 g/day, a 61% increase in stone formers consuming 1 g/day and a 75% increase in stone formers consuming 2 g/day[56]. It is possible that more AA breaks down to oxalate in stone formers due to the increased oxidative stress associated with the disease[57].

In 2008 Taylor and Curhan reported on factors that influenced urinary oxalate excretion in over 3,000 participants who submitted 24 hr urine samples as part of their epidemiological studies of stone formation in male health professionals and female nurses[14]. Approximately two thirds had a history of kidney stone formation. Of the dietary factors analyzed, which included calcium, oxalate, animal protein, vitamin B6 and AA, and urinary excretions of potassium, sodium, and phosphorus which reflect the intake of these minerals, AA had the greatest effect on urinary oxalate excretion. Compared with participants consuming < 90 mg/day, participants with an intake > 1000 mg/day had a urinary oxalate excretion that was 6.8 mg/day higher when adjusted for confounding variables. This was much greater than the effect of dietary calcium which reduced urinary oxalate by only 2.3 mg/day in an analysis of quartile groups of intake with similar adjustments. These results alone suggest that calcium oxalate stone formers should cease taking supplemental AA should decrease their intake of AA-rich foods. Clearly the effects of AA intake warrant much closer scrutiny for its influence on urinary oxalate excretion and calcium oxalate stone formation.

## PARENTERAL AND ENTERAL SOURCES OF ASCORBIC ACID AND URINARY OXALATE EXCRETION

Individuals receiving parenteral and enteral fluids to meet their nutritional needs require a source of AA. Due to the lability of AA, care has to be taken that it does not breakdown to oxalate during preparation or storage. Ribeiro et al have reported that 15% of the AA in a pediatric parenteral formulation was lost after storage for 3 days at 25 °C[58]. This loss depended on the storage temperature, storage time and photoprotection. We have previously suggested that the oxalate we detected in the defined formula diet, Ensure®, is derived from AA during its preparation[59]. The Food and Drug Administration requires that 200 mg AA be added to multivitamin preparations used in parenteral solutions to meet daily nutritional requirements. Pena de la Vega et al found that the increase from 100 – 200 mg/d was associated with a 30% increase in urinary oxalate excretion[60]. Despite this increase in oxalate excretion, studies have not yet associated it with an increased stone incidence in individuals receiving parenteral nutrition.

## ASCORBIC ACID INGESTION AND OXALATE NEPHROPATHY

There have been a number of reports of oxalate nephropathy that suggest the oral ingestion or intravenous administration of moderate to large amounts of AA was a contributing factor (Table 2). The majority were treated with dialysis and in all but two, dialysis was temporary. Two of the reported cases resulted in mortality. Intratubular calcium oxalate deposits were demonstrated in all of the renal biopsies and other findings included mesangial hypercellularity, tubular atrophy, interstitial fibrosis, tubulointerstitial nephritis and edema. A number of these subjects took mega-doses of AA in hopes that it would improve their general health or provide relief or cure of ongoing diseases. This approach was popularized a number of years ago by Noble Prize recipient, Dr. Linus Pauling[61]. The benefits of consuming large amounts of AA have not been substantiated and the risk associated with an increased oxalate production is substantial. AA has been demonstrated to have anti-proliferative and anti-tumor effects in cell culture and animal models. To achieve cytotoxic concentrations of AA some investigators have administered large doses of intravenous AA to patients afflicted with advanced cancer[13, 40]. While oncologic benefits have not yet been proven, patients have experienced limited toxicity in trials of a short duration[40]. However, such infusions could potentially result in oxalate nephropathy. The public needs to be informed of this risk as there is an increasing pool of candidates receiving large doses of intravenous AA under the guidance of complementary and alternative medicine practitioners for purported health benefits[62]. The patient in Table 2 administered the unspecified dose of AA IV received it in such a clinic[63]. Over 30,000 individuals may have received such infusions with an average of 22 treatments per patient for the treatment of infection, fatigue, cancer and other conditions with unknown consequences. An assessment of whether there is any decline in kidney function associated with these treatments is warranted.

## CONCLUSION

Epidemiological evidence indicates that the amount of AA ingested is a risk factor for calcium oxalate stone disease and that this risk is associated with the amount of oxalate

excreted in urine. Short term experiments with human subjects have confirmed that supplemental AA increases urinary oxalate excretion. The available evidence presented above indicates that the oxidized form of AA, DHA, can form an open chain isomer, DKA, which is unstable and breaks down to oxalate and 4-carbon sugars. What is not clear is how much AA and DHA is metabolized in cells and tissues, and how much is converted to DKA and ultimately oxalate. Each of these pathways may be influenced by the diet, physiological effectors or by genetic variation. How the amount converted to oxalate is modulated is important knowledge to accrue to decrease endogenous oxalate synthesis and to identify optimal intake levels for individuals with calcium oxalate stone disease. While our investigations on the sources of endogenous oxalate synthesis indicate that hydroxyproline metabolism may account for 30 – 40%, it remains possible that AA is also a major source. Finally, it is clear that compromised renal function coupled with excessive AA ingestion can result in oxalate nephropathy in susceptible individuals.

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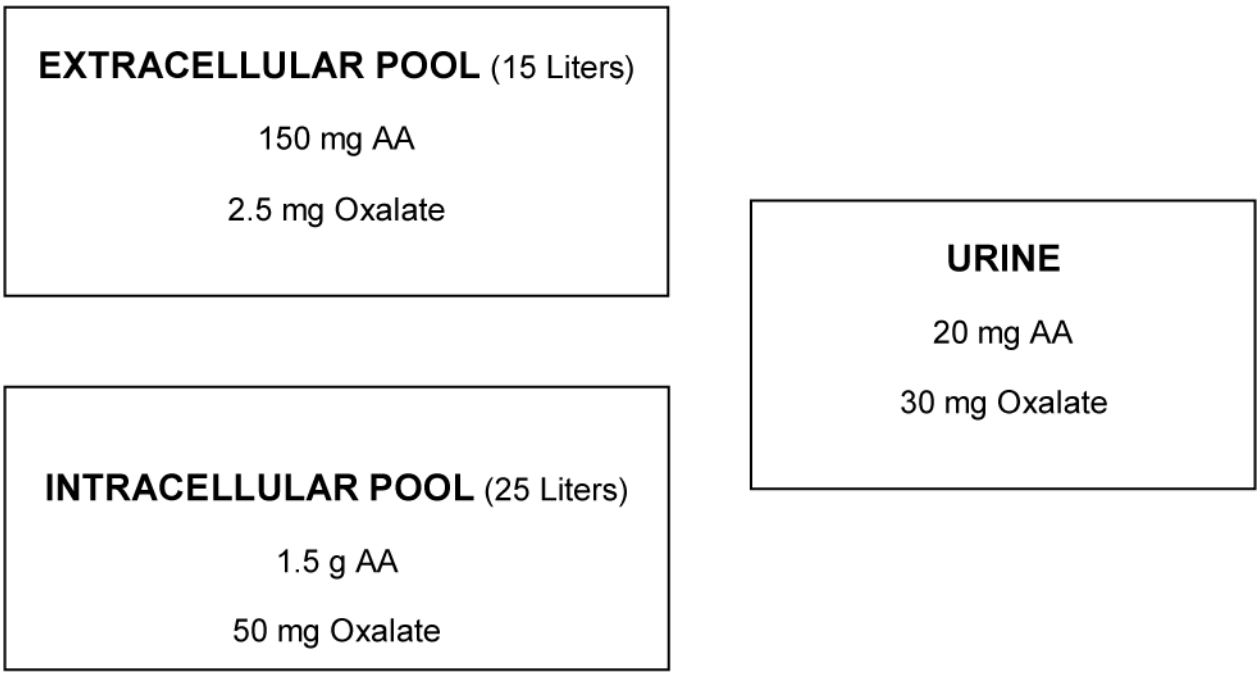


**Figure 1.**
Estimated average compartment sizes of the AA and oxalate pools in humans.

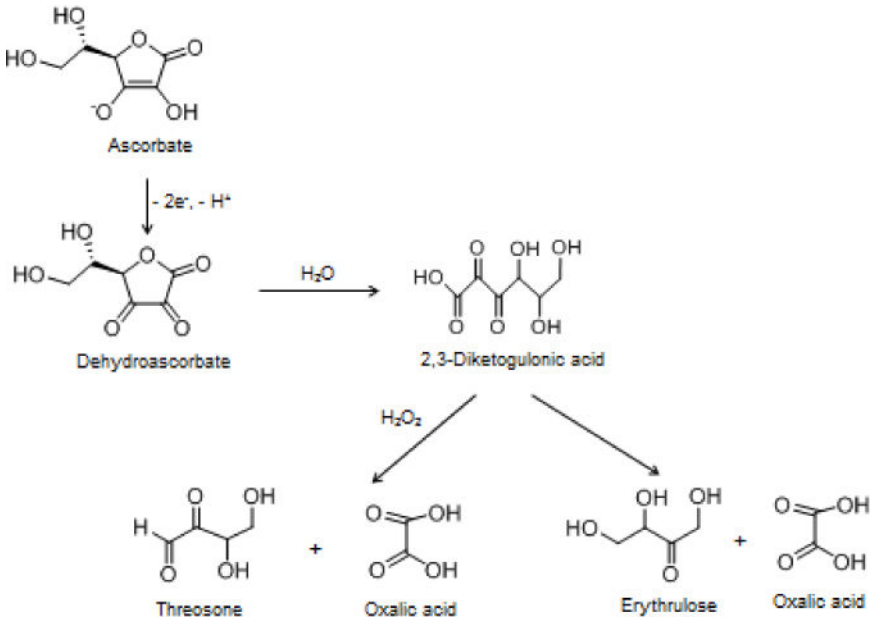


**Figure 2.**
Major products in AA degradation pathway.

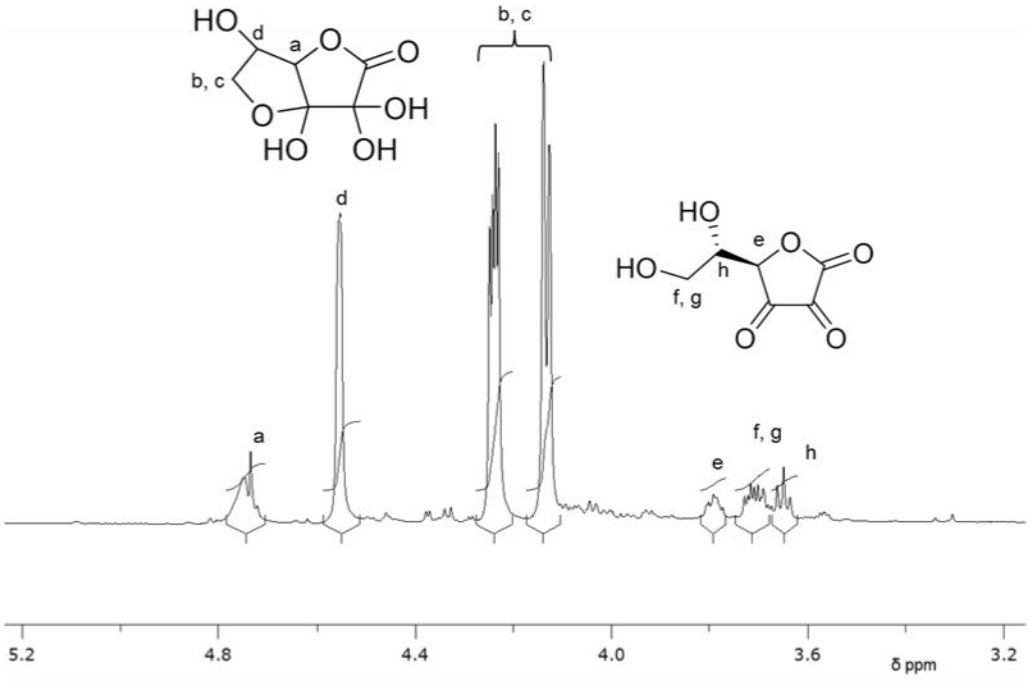


**Figure 3.**

$^{1}$HNMR spectrum (Bruker 850 Mhz NMR spectrometer) of DHA (3 mM) in $D_2O$, showing proton assignments for hydrated bicyclic (a–d) and open chain (e–h) forms.

**TABLE 1**

Vitamin C Content of Selected Raw Fruits and Vegetables

| Food | Total Vitamin C (mg/100g) |
|---|---|
| Broccoli | 89.2 |
| Chili Pepper Green | 242.5 |
| Cauliflower | 48.2 |
| Guava | 228.3 |
| Kiwi Fruit | 92.7 |
| Lemon Peeled | 53.0 |
| Orange Peeled | 53.2 |
| Pepper Green | 80.4 |
| Pepper Red | 127.7 |
| Parsley | 133.0 |
| Strawberry | 58.8 |

Values taken from the USDA Database (http://ndb.nal.usda.gov/ndb/search/list)

**Table 2**

Vitamin C ingestion and oxalate nephropathy

| Ref. | Sex | Age | Reason for adminstration | Amount | Highest serum Cr mg/dL | Dialysis | Final serum Cr mg/dL |
|---|---|---|---|---|---|---|---|
| 64 | M | 73 | Patient choice | 680 mg/dy High Ox diet | 8.4 | Y Temp | 1.8 |
| 65 | M | 51 | Metastatic prostate cancer | 60 g IV | 13.3 | N | 2.9 |
| 63 | F | 51 | Lupus | 2 IV doses (Amount ?) | 7.2 | Y Temp | 1.1 |
| 8 | F | 58 | Nephrotic Syndrome | 45 g IV | 3.5 | Y | Death |
| 12 | M | 58 | Burn injury followed by parenteral nutrition | 1.6 g/dy for 50 dys | | Y Temp | 1.4 |
| 11 | M | 31 | Patient choice | 2 – 5 g/dy several dys | 10.1 | Y Temp | 2.2 |
| 10 | F | 49 | Patient choice | 4 g/dy several mths | 4.5 | N | 1.1 |
| 9 | M | 72 | Patient choice | 480 – 960 mg/dy several mths | 10.6 | Y Temp | 1.9 |
| 66 | M | 72 | Patient choice | Several grams daily (Period ?) | 15 | Y | Death |
| 67 | F | 58 | Patient choice | 3 – 6.5 g/dy for 1 mth | 9.1 | Y | Awaiting transplant |