Higher doses of ascorbic acid may have the potential to promote nutrient delivery via intestinal paracellular absorption

Ivana Roosevelt Sequeira

ORCID number: Ivana Roosevelt Sequeira 0000-0001-5414-9925.

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Abstract

The significance of plasma ascorbic acid (AA) is underscored by its enzymatic and antioxidant properties as well as involvement in many aspects of health including the synthesis of biomolecules during acute illness, trauma and chronic health conditions. Dietary intake supports maintenance of optimal levels with supplementation at higher doses more likely pursued. Transient increased intestinal paracellular permeability following high dose AA may be utilised to enhance delivery of other micronutrients across the intestinal lumen. The potential mechanism following dietary intake however needs further study but may provide an avenue to increase small intestinal nutrient co transport and absorption, including in acute and chronic illness.

Key Words: Aspirin; Ascorbic acid; Paracellular permeability; Antioxidant; Lactulose mannitol test

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Core Tip: The significance of plasma ascorbic acid (AA) is underscored by its enzymatic and antioxidant properties as well as involvement in many aspects of health including the synthesis of biomolecules during acute illness, trauma and chronic health conditions. Dietary intake supports maintenance of optimal levels with supplementation at higher doses more likely pursued. Transient increased intestinal paracellular permeability following high dose AA may be utilised to enhance delivery of other micronutrients across the intestinal lumen. The potential mechanism following dietary intake however needs further study but may provide an avenue to increase small intestinal nutrient co transport and absorption, including in acute and chronic illness.
Ascorbic acid (AA) or Vitamin C is an essential micronutrient in the human diet, due to the non-functional gene encoding L-glucono lactone oxidase that is involved in the catalysis of the last biosynthetic step in the pathway. The physiology and pathophysiology of AA has been extensively studied and the estimated plasma half-life reported to be between 7-14 d on the basis of depletion-repletion studies, conducted in healthy male subjects. Ergo, providing an approximation of the plasma half-life in those individuals that may have acute illness, trauma or chronic health conditions. The importance of maintaining optimal AA levels is underscored by both its antioxidant and non-antioxidant, i.e., enzymatic roles and involvement in many aspects of health which include being an essential factor in the synthesis of collagen, carnitine and norepinephrine. Reduced dietary consumption, due to lower intake of fresh fruit and vegetables, or increased utilization via intracellular uptake are important aspects in the maintenance of plasma AA levels. Increased cellular distribution of AA has been shown to be associated with high leukocyte turnover and oxidative stress, whereby the redox potential of AA mitigates the production of reactive oxygen species (ROS). Dehydroascorbic acid (DHA), formed by the loss of electron by the ascorbic radical, has the same biological activity as the reduced form. The subsequent hydrolysis of DHA, if not reduced back to AA, to 2,3-diketogulonic acid is an irreversible step that results in the loss of antioxidant properties and its degradation.

Exogenous AA uptake is provided by, but not limited to, dietary intake. Due to AAs proposed antioxidant as well as enzymatic health benefits it is a widely sought-after dietary supplement that is available for consumption alone or as a multivitamin. Notably, AA has been co-formulated not only with other vitamins but also with anti-inflammatory pharmacological agents[1-3], in particular with aspirin. Monotherapy with aspirin has been shown to decrease the concentration of AA not only within the intestinal enterocytes[4] but also in gastric juice[5] potentiating negative impacts by reducing the ability of intestinal mucosal cells to manage oxidative stress[4]. Therefore, the co-formulation of AA with aspirin is considered to mitigate and ameliorate ROS induced gastrointestinal damage that has been implicated with the etiology of aspirin monotherapy[6].

Of interest is, a body of work conducted by our group that demonstrated that a single dose of aspirin has the potential to cause transient increases in small intestinal permeability over a 6 h period in healthy individuals. More importantly that rather than mitigating the aspirin induced increase in intestinal permeability[7], assessed by the lactulose mannitol test[8], the administration of 500 mg AA augmented absorption of lactulose either when given alone or in combination with the single 500 mg dose of aspirin (Table 1).

Therefore, it appears that while simultaneous dosage of AA with aspirin may potentially have longer term beneficial effects, related to the antioxidant properties of AA, in the short term however it may not mitigate the aspirin-induced increase in intestinal permeability. It is notable that, in the investigation, participants received an aspirin drink thirty minutes prior to the AA drink[7] in contrast to other studies where AA and aspirin were administered together as a single solution or in the form of a tablet[1-3,5,9]. Hence, it may be considered that the extent of ionization of each of these weak organic acids could have differed in the intestinal lumen consequently affecting their ability to access transporters in the gut wall. The proportion of unionized AA increases to 99% and 15% in the stomach (pH 1) and small intestine (pH 5) respectively, and under these conditions passive diffusion is thought to play a significant role in AA uptake[10]. Indeed, it has been previously shown that co-administration of aspirin with AA decreased the rate at which AA was absorbed in vivo[11] and could have resulted from interference with sodium dependent secondary active transport via sodium-dependent vitamin C transporters (SVCT1 and SVCT2) [12]. These transporters are differentially expressed along the length of the gastrointestinal tract, with the pattern of expression mediated in part by transcriptional and epigenetic mechanisms[13].
Pertinent however is that increased small intestinal mucosal permeability, even if transient, following dosage with AA raises questions as to whether similar changes may occur following dietary ingestion of foods that are rich in AA. It is noteworthy that the dose of 500 mg AA administered in our study[7] is higher than the recommended daily allowance (RDA 60-120 mg)[14], guidelines for which are variable. An increase in the RDA to 200 mg has been proposed[15] to maximize the attributed health benefits, with evidence suggesting that regular supplement users consume more than 1 g AA per day[16]. Whether the increase in intestinal permeability detected in our study was dose dependent is not known and a comparison with other publications is not possible as the effect of AA on intestinal permeability has not been previously studied. We hypothesize that the dose of AA induced increase in intestinal permeability could be due to its action on apical transporters. Glucose has been shown to modulate vitamin C transport at the small intestinal brush border membrane with similar rates of uptake of DHA and AA reported in the absence of glucose[17]. The reduced form, AA, is absorbed via SVCT1 and SVCT2[18]. Given that the stoichiometry of the SVCTs are similar to those of the sodium dependent glucose transporter (SGLT1)[19,20] it is possible that absorption of AA may bring about intracellular changes that modulate, i.e., relax, tight junctions in a manner similar to that of glucose transport via SGLT1[21]. Additionally, the oxidised form DHA has been shown to compete with glucose for transport via glucose transporters[22] in particular GLUT2 and GLUT4[23], which have been suggested along with SGLT1 to cause cytoskeletal contraction[24]. The modulation of the tight junctions in this manner has been shown to increase mucosal permeability[25], allowing greater quantities of larger molecules to be absorbed via the paracellular pathway[26].

Relating and extending the results of our study to AA levels from dietary intake maybe difficult and may necessitate human intervention studies using whole foods or extracts at comparable doses. However, an extrapolation of the effect of foods/extract would require careful study design as the amount of available AA in different foods may vary widely[27]. Particularly in fruits and vegetables where the quantity of AA is determined by a variety of factors which include cultivars, environmental conditions including regional and seasonal conditions as well as maturation[28]. Additionally, depending on the levels of hydration it is very likely that there may be inherent variability in the proportion of reduced and oxidized AA within fruits and vegetables, much like AA in solutions which have a greater susceptibility to oxidation. At pH < 4 [29] the concentration of AA deteriorates, with greater losses shown to occur in frozen products that are processed/canned [mean: 26 % (0% - 78 %)] than from frozen fresh products [mean: 18 % (0% - 50 %)][30]. Furthermore, supplementation of products with synthetic AA has been shown to facilitate oxidation within products[31].

The degradation of AA not only occurs during processing and storage of the food/extract but also once it is consumed and introduced into the gastrointestinal lumen. Being an antioxidant, AA gets oxidized in the gastrointestinal tract to maintain the reduced state of other nutrients, e.g., iron or form metal-oxygen-ascorbate complexes[32]. However, the acidic conditions of the gastrointestinal tract protect AA against chemical and enzymatic oxidation[32] with 93% of AA from bioactive broccoli inflorescence[33] and 71% from pomegranate juice[34] shown to be stable following in vitro gastric digestion. Conversely, in the small intestinal environment greater amounts

### Table 1 Variation in the cumulative excretion of lactulose in bulked urine samples collected over two time periods (first three hours and the second three hours) during a six hours collection period in 28 healthy female participants

| Treatment            | First 3 h | Second 3 h |
|----------------------|-----------|------------|
| Aspirin              | 0.37 ± 0.05<sup>a,b</sup> | 0.46 ± 0.05<sup>c</sup> |
| Ascorbic acid        | 0.47 ± 0.05<sup>c</sup>   | 0.53 ± 0.04  |
| Combined dosage      | 0.68 ± 0.09<sup>c</sup>   | 0.77 ± 0.14<sup>d</sup> |

<sup>a</sup>Statistically significant (P < 0.05) differences between treatments during the first 3 h, results expressed as mean ± SEM.
<sup>b</sup>Statistically significant (P < 0.05) differences between treatments during the second 3 h, results expressed as mean ± SEM.
<sup>c</sup>Statistically significant (P < 0.05) differences between treatments during the second 3 h, results expressed as mean ± SEM.
<sup>d</sup>Statistically significant (P < 0.05) differences between treatments during the second 3 h during the lactulose mannitol test, results expressed as mean ± SEM.
of AA was oxidized, with 39% of AA recovered\textsuperscript{[35]} in this segment of the gut following \textit{in vitro} small intestinal digestion of blended fruit juice. While this information is pertinent to our understanding, the bioavailability of luminal AA also requires consideration of the interaction with other contained dietary constituents \textit{e.g.}, fiber and other bioflavonoids. Micronutrients within fruit juice have been shown to inhibit the absorption of AA\textsuperscript{[36]} at doses between 50-500 mg. One mechanism for the inhibition is thought to occur \textit{via} competition with transporters which has been particularly attributed to flavonoids such as quercetin and myricetin that are shown to decrease ascorbate as well as DHA absorption in \textit{in vitro} models\textsuperscript{[37,38]}. Vinson \textit{et al}\textsuperscript{[39]} further demonstrated in guinea pigs that dosage with a citrus juice extract, containing 18% bioflavonoids, 15% proteins and 30% carbohydrates, resulted in a significantly slower rise in plasma ascorbate concentrations in comparison to a simple ascorbate solution. In alignment with these findings, a similar effect was also reported in human participants following the intake of citrus fruit extract\textsuperscript{[40]}.

Contrary to these findings it has also been shown that flavonoids, following consumption of half a golden kiwi fruit a day (50 g) over six weeks, did not alter the bioavailability of contained AA when compared to 50 g of synthetic AA\textsuperscript{[41]}. This inferred effect appeared consistent as the administration of AA with blackcurrant juice\textsuperscript{[42]} or orange juice\textsuperscript{[43]} containing flavonoids did not impede absorption. Therefore, it seems likely that these differences in the manner in which AA is absorbed when co-administered could have arisen due to the differential gastric transit times\textsuperscript{[44]}. Gastric emptying of liquid meals or beverages is dependent on the pressure gradient across the gastroduodenal junction and in part by the composition of the duodenal content, and hence it is conceivable that the nutrient content and composition of these different solutions or fruit slowed down gastric emptying\textsuperscript{[44]}. Alternatively, these incongruent results may in part have been due to the different forms and dosages at which AA was administered, \textit{i.e.}, as solutions or gels. Ergo resulting in potentially variable gastrointestinal residence and emptying times to influence absorption and the subsequent appearance of AA in sampled biological fluids \textit{i.e.}, plasma, urine etc.

Given these inconsistencies in evidence regarding the bioavailability when co-administered with other substances, it remains unclear as to whether the effects of AA on intestinal permeability can be extended to situations when it is delivered in foods. This in particular considering the dose and quantity of the foods/extract that would have to be consumed to show comparable effects. All the same, the ability of a pharmaceutical dose of 500 mg AA to augment absorption of larger inert sugars such as lactulose does raise the possibility that AA could be a putative agent that could be co-administered with poorly absorbed drugs, nutrients or food substances of similar or larger molecular weight to enhance their absorption\textsuperscript{[45,46]}.

Increased paracellular permeability, \textit{via} the modulation of tight junctions, has been reported after consumption of a meal especially in the presence of glucose\textsuperscript{[47]} and alanine\textsuperscript{[48]}. Using Caco 2 models, food components such as plant extracts\textsuperscript{[49-51]} and isolated food components\textsuperscript{[52-54]} have been shown to increase paracellular permeability with the resultant flux in macromolecules, such as mannitol\textsuperscript{[51,55,56]}. These authors, including Kosińska \textit{et al}\textsuperscript{[57]}, postulated that food components could be safe alternatives to reversibly ‘open’ tight junctions in order to enhance the absorption of molecules of interest. Conversely, glutamine, polyunsaturated fatty acids and polyphenols have been shown to ‘tighten’ tight junctions through increased expression of tight junction associated proteins\textsuperscript{[58]}.

**CONCLUSION**

Whilst the molecular mechanism by which AA increases paracellular permeability remains to be elucidated, a dose of 500 mg, which is higher than the current RDA, has been shown to increase small intestinal permeability in healthy individuals. Whether this effect is dose dependent requires further exploration however together with its antioxidant properties, the alteration of intestinal permeability by AA can be potentially explored as a safe and novel application for the delivery of molecules \textit{via} the paracellular pathway. Indeed it is also conceivable the co-administration of AA, as a supplement or through consumption of a rich food source, with aspirin may potentiate its serum levels and analgesic effects.
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