Nutrients 2013, 5, 4284-4304; doi:10.3390/nu5114284

Review

Synthetic or Food-Derived Vitamin C—Are They Equally Bioavailable?

Anitra C. Carr * and Margreet C. M. Vissers

Centre for Free Radical Research, Department of Pathology and Biomedical Science, University of Otago, Christchurch, P.O. Box 4345, Christchurch 8140, New Zealand; E-Mail: margreet.vissers@otago.ac.nz

* Author to whom correspondence should be addressed; E-Mail: anitra.carr@otago.ac.nz; Tel.: +64-3-378-6498; Fax: +64-3-378-6540.

Received: 30 August 2013; in revised form: 22 September 2013 / Accepted: 14 October 2013 / Published: 28 October 2013

Abstract: Vitamin C (ascorbate) is an essential water-soluble micronutrient in humans and is obtained through the diet, primarily from fruits and vegetables. In vivo, vitamin C acts as a cofactor for numerous biosynthetic enzymes required for the synthesis of amino acid-derived macromolecules, neurotransmitters, and neuropeptide hormones, and is also a cofactor for various hydroxylases involved in the regulation of gene transcription and epigenetics. Vitamin C was first chemically synthesized in the early 1930s and since then researchers have been investigating the comparative bioavailability of synthetic versus natural, food-derived vitamin C. Although synthetic and food-derived vitamin C is chemically identical, fruit and vegetables are rich in numerous nutrients and phytochemicals which may influence its bioavailability. The physiological interactions of vitamin C with various bioflavonoids have been the most intensively studied to date. Here, we review animal and human studies, comprising both pharmacokinetic and steady-state designs, which have been carried out to investigate the comparative bioavailability of synthetic and food-derived vitamin C, or vitamin C in the presence of isolated bioflavonoids. Overall, a majority of animal studies have shown differences in the comparative bioavailability of synthetic versus natural vitamin C, although the results varied depending on the animal model, study design and body compartments measured. In contrast, all steady state comparative bioavailability studies in humans have shown no differences between synthetic and natural vitamin C, regardless of the subject population, study design or intervention used. Some pharmacokinetic studies in humans have shown transient and small comparative differences between synthetic and natural vitamin C, although these differences are likely
to have minimal physiological impact. Study design issues and future research directions are discussed.

**Keywords:** ascorbate; dietary vitamin C; bioavailability; animal studies; human studies; bioflavonoids

---

### 1. Introduction

Vitamin C (ascorbate) is an essential water-soluble micronutrient in humans and is obtained through the diet primarily from fruits and vegetables [1]. *In vivo*, it acts as a cofactor for numerous biosynthetic enzymes required for the synthesis of amino acid-derived macromolecules, neurotransmitters and neuropeptide hormones [2], and for various hydroxylases involved in the regulation of gene transcription and epigenetics [3,4]. Vitamin C is concentrated from the plasma into the body’s organs and is found in particularly high concentrations in the pituitary and adrenal glands and in the corpus luteum [5], although skeletal muscle, brain, and liver comprise the largest body pools [6]. Most animals can synthesize vitamin C from glucose in the liver [7]; however, humans and a small selection of animal species have lost the ability to synthesize vitamin C due to mutations in the gene encoding L-gulono-γ-lactone oxidase, the terminal enzyme in the vitamin C biosynthetic pathway [8]. Therefore, an adequate and regular dietary intake is essential to prevent hypovitaminosis C and the potentially fatal deficiency disease, scurvy [9].

In the mid 1700s the Royal Navy surgeon James Lind carried out controlled dietary trials and determined that citrus fruit could cure individuals with scurvy (reviewed in [10]). However, it wasn’t until the early 1900s that experimental scurvy was first produced in guinea pigs through dietary restriction and shown to be prevented by feeding the animals fresh fruits and vegetables. In the early 1930s vitamin C was isolated from fruit and vegetables and adrenal cortex and was named “hexuronic acid”, which was shown to cure scurvy in guinea pigs and was subsequently renamed ascorbic acid to reflect its anti-scorbutic properties. Vitamin C was first chemically synthesized in 1933 [10] and, since the mid 1930s, the question of the comparative bioavailability of synthetic versus natural, food-derived vitamin C in animal models and human subjects has been a point of consideration.

The bioavailability of dietary vitamin C represents the proportion of the micronutrient that is absorbed by the intestines and is available for metabolic processes within the body. *In vivo* vitamin C levels are a function of uptake, metabolism, and excretion (see [11] for an excellent review of these processes). Vitamin C is actively transported into the body via two sodium-dependent vitamin C transporters, SVCT1 and SVCT2 [12,13]. These transporters exhibit different tissue distributions and uptake kinetics. SVCT1 is expressed in epithelial tissues and is primarily responsible for intestinal uptake and renal reabsorption of vitamin C, the latter helping to maintain whole body homeostasis [13]. SVCT2 is expressed in specialized and metabolically active tissues and is required for delivery of vitamin C to tissues with a high demand for the vitamin either for enzymatic reactions [2] and/or to help protect these tissues from oxidative stress [13]. Both of these transporters show significantly more affinity for the L- *versus* D-isoform of vitamin C (Figure 1) [12,14], and this selectivity likely explains earlier observations of significantly lower tissue accumulation and anti-scorbutic activity of D-ascorbic
acid in guinea pigs [15,16]. Although D-ascorbic acid is a commonly added food preservative [17], administration of D- and L-ascorbic acid together does not affect the bioavailability of the latter in humans [18].

Through its action as a reducing agent and antioxidant, ascorbate undergoes one and two electron oxidations to produce the ascorbyl radical and dehydroascorbic acid (DHA) (Figure 1). Recent research has shown that DHA can be taken up by the facilitative glucose transporters GLUT2 and GLUT8 in the small intestine [19]. Cells are also able to transport DHA via GLUT1 and GLUT3 [20,21], followed by intracellular reduction to ascorbate [22,23]. However, transport via the GLUTs is in competition with glucose which is at relatively high concentrations throughout the body and although different fruits and vegetables have been shown to contain relatively high amounts of DHA [24], the \textit{in vivo} contribution of DHA is uncertain due to its minimal circulating and organ levels [25,26] (although white blood cells may be an exception to this) [27,28].

\textbf{Figure 1.} Vitamin C in its reduced form (ascorbic acid), shown as both its L- and D-isomers, and its two electron oxidation form (dehydroascorbic acid, DHA). DHA can be readily reduced back to ascorbic acid \textit{in vivo} via both chemical and enzymatic pathways [23].

Synthetic and food-derived vitamin C is chemically identical. However, fruit and vegetables are rich in numerous micronutrients (vitamins and minerals), dietary fiber, and phytochemicals (e.g., bioflavonoids), and the presence of some of these may affect the bioavailability of vitamin C. Vitamin C has long been known to interact with vitamin E by reducing the tocopheroxyl radical and regenerating native tocopherol [29]. Some fruit, such as kiwifruit, contain relatively high amounts of vitamin E and one animal study has indicated that vitamin E is able to preserve vitamin C \textit{in vivo} [30]. Food-derived (and synthetic) vitamin C is well known to increase non-heme iron uptake and body status, likely via its ability to reduce iron from its ferric to ferrous state [31,32]. However, whether iron can affect vitamin C bioavailability is less clear [33–35]. Although iron has been shown to increase the uptake of vitamin C in cultured intestinal cells [33], human intervention studies have shown no effect of iron intake on vitamin C bioavailability [34,35]. One study has indicated that specific dietary fibers, such as hemicellulose and pectin, may affect the excretion of vitamin C [36], however, their influence on vitamin C uptake was not determined.

Plant-derived flavonoids have been of interest since the mid 1930s, when they were initially referred to as “vitamin P”, primarily due to their effect on vascular permeability [37]. At the time, there was much debate in the literature regarding the role of “vitamin P” in experimental [38–42] and human scurvy [37,43–45]. Flavonoids can act as antioxidants via direct scavenging of free
radicals [46,47] and/or chelation of redox-active metal ions [48,49]. As a result, it has been suggested that flavonoids may “spare” vitamin C and, thus, increase its bioavailability. Flavonoids have been shown to inhibit the in vitro oxidation of vitamin C [48–51], however, the in vivo relevance of metal-ion mediated oxidation of vitamin C is likely to be minimal as free metal ions are largely sequestered in the body [52]. Whether flavonoids can affect vitamin C uptake in vivo is uncertain due to the low plasma bioavailability of these compounds [53]. Thus, any interaction of flavonoids with vitamin C would be expected to occur primarily in the intestinal lumen prior to active uptake.

Of note, several in vitro studies have shown that various flavonoids can inhibit vitamin C and DHA uptake by their respective transporters. The flavonoid quercetin can reversibly inhibit SVCT1 expressed in Xenopus oocytes [54] and limited data from an animal model indicates that this may occur in vivo [54]. Quercetin and myricetin can inhibit the uptake of vitamin C and DHA into cultured monocytic (HL-60 and U937) and lymphocytic (Jurkat) cells via inhibition of GLUT1 and GLUT3 [55] and possibly also SVCT2, which is expressed in leukocytes [56]. Quercetin and phloretin can also inhibit the intestinal GLUT2 and GLUT8 transporters [19]. Thus, based on the above in vitro studies, it is unclear whether flavonoids will enhance in vivo vitamin C bioavailability through a sparing action, or decrease its bioavailability through inhibiting vitamin C transporters.

The effect of various purified flavonoids or flavonoid-rich fruits and vegetables on vitamin C bioavailability in different animal models and human subjects is discussed below. To test comparative vitamin C bioavailability, both steady-state and pharmacokinetic models have been used. The former monitors ascorbate levels in blood and/or urine following a number of weeks of supplementation, while the latter monitors transient changes in plasma levels and/or urinary excretion over the hours following ingestion of the vitamin C-containing test substance. The gold standard for analysis of vitamin C is HPLC with coulometric electrochemical detection due to its sensitivity and specificity [57]. Early studies, however, were limited primarily to colourimetric methods based on reduction of ferric iron compounds and are prone to interference by numerous other substances [57].

2. Vitamin C Bioavailability Studies Using Animal Models

There are a number of benefits to the use of animal models to investigate vitamin C bioavailability, particularly the ease of diet control and the ability to obtain tissues not normally accessible in human studies. However, results can vary widely depending on the animal model used and the different treatment and analytical methodologies employed. It should also be noted that not all of the animal models that have been used are naturally vitamin C deficient. The animal models of choice are the naturally vitamin C deficient guinea pig, and genetically scurbutic animal models, such as the Osteogenic Disorder Shionogi (ODS) rat [58], the L-gulono-γ-lactone oxidase (Gulo−/−) knockout mouse [59], and the spontaneous bone fracture (sfx) mouse [60]. Although animal studies can provide useful information, translation of the findings to humans should always proceed with caution.

Studies investigating the comparative bioavailability of synthetic versus natural vitamin C in animal models are shown in Table 1. Studies carried out in guinea pigs showed enhanced uptake of vitamin C into specific organs (e.g., adrenals and spleen) in the presence of flavonoid-rich juices/extracts or purified plant flavonoids (e.g., hesperidin, rutin, and catechin) [42,61–64]. Vinson and Bose [65] carried out a pharmacokinetic study in guinea pigs and found a 148% increase in the area under the
plasma ascorbate concentration-time curve when administered as citrus fruit media. They also noted that the citrus fruit group demonstrated delayed plasma vitamin C uptake compared with the synthetic vitamin C group [65]. Cotereau et al. [42] reported that animals given both vitamin C and catechin not only had four to eight-fold more vitamin C in the organs measured, but they were also the only group without scorbutive-type lesions. The latter finding was supported by a similar study showing fewer fresh hemorrhages in scorbutic guinea pigs receiving vitamin C with rutin or querceitin compared with vitamin C alone [66].

Several of the studies in Table 1, however, showed no differences in vitamin C accumulation in some organs (e.g., liver) [61–63,67]. Hughes et al. noted that the acerola cherry preparation they used was virtually flavonoid free due to dilution of the high vitamin C fruit extract, which they suggested may have accounted for its reduced efficacy compared with blackcurrant juice, which is flavonoid rich [64]. To account for the flavonoid-dependent differences in vitamin C uptake observed between the adrenals and livers of guinea pigs [62,63], Douglass and Kamp [62] noted that flavonols such as rutin are rapidly destroyed in liver tissue, but are relatively stable in adrenal homogenates. Papageorge et al. [63] also noted that when epinephrine oxidizes it can contribute to the destruction of vitamin C and thus the antioxidant effects of rutin may result in “sparing” of vitamin C in adrenals. A study by Levine’s group [54] showed that the flavonoid quercetin can reversibly inhibit vitamin C intestinal transport and decrease plasma levels of the vitamin in the CD (Sprague-Dawley) rat, although it should be noted that this is not a vitamin C deficient animal model. Some of the variability observed in these different animal studies (Table 1) may be due to the varying ratios of flavonoid to vitamin C employed.

We recently carried out a comparative bioavailability study, using the Gulo mouse model, investigating the uptake of vitamin C from kiwifruit gel compared with synthetic vitamin C [68]. We found that the kiwifruit extract, which is rich in flavonoids [69,70], provided significantly higher serum, leukocyte, heart, liver, and kidney levels of vitamin C than the purified vitamin, suggesting some type of synergistic activity of the whole fruit in this model. As with Wilson et al. [61], we did not observe any difference between the two interventions with respect to vitamin C uptake into the brain. Indeed, there is significant retention of vitamin C in the brain during dietary depletion [64,68], suggesting a vital role for vitamin C in the brain. Thus, a significant proportion of animal studies show enhanced circulating and organ levels of vitamin C in the presence of food-derived or purified flavonoids.

3. Steady State Bioavailability Studies in Humans

An early report of several patients with scurvy whose plasma vitamin C levels did not increase with synthetic vitamin C alone, but only in the form of lemon juice [45], initially lent support to the “vitamin P”/flavonoid theory. However, in contrast to the animal studies, all steady state human studies (summarized in Table 2) have shown little difference in plasma and/or urine bioavailability between synthetic vitamin C and that from different fruits, fruit juices, and vegetables [35,71–76]. Mangels et al. [35] did observe a 20% lower plasma bioavailability of vitamin C from raw broccoli compared with cooked broccoli, however, this may have been due to differences in mechanical homogenization (chewing), a similar effect to that observed for carotenoid absorption from raw versus cooked carrots.
We recently carried out a steady state bioavailability study in young non-smoking men supplemented for six weeks with 50 mg/day vitamin C, in the form of a chewable vitamin C tablet or half a gold kiwifruit [77]. This dose was chosen as it lies on the steep part of the sigmoidal plasma uptake curve [78], thus enhancing the likelihood of detecting a difference between the two interventions. Although most steady state studies have used sequential or crossover study designs, we chose a randomized parallel arms design for a number of reasons. Block et al. [79] have previously observed a lower plasma vitamin C response to supplemental vitamin C in the second phase of a multiple depletion/repletion study. Furthermore, although washout of vitamin C could be monitored between the two phases of a cross-over study, it would not be possible to monitor washout of other kiwifruit-derived components, e.g., vitamin E, which may affect the second phase of a cross-over study due to potential in vivo interactions with the supplemental vitamin C [30].

Only one previous study has investigated the comparative bioavailability of synthetic versus natural vitamin C in leukocytes [71]. These investigators found no difference in leukocyte vitamin C uptake between synthetic vitamin C (in the presence or absence of rutin) and that in orange juice two hours after a single 75 mg dose [71]. Therefore, in addition to plasma, urine, and semen samples, we also isolated peripheral blood mononuclear cells and neutrophils before and after intervention. Due to ease of accessibility and isolation, peripheral blood leukocytes are often used as a marker for tissue vitamin C status, however, whether they are a good model for all organs and tissues is uncertain. In support of this premise our animal study indicated that different organs exhibited maximal vitamin C uptake at varying doses of the vitamin [68] and we have recently shown that human skeletal muscle exhibits greater relative uptake of vitamin C than leukocytes [80]. Therefore, we also carried out needle biopsies of skeletal muscle tissue (vastus lateralis), before and after intervention. In contrast to our earlier animal study [68], our human study clearly showed no differences in the steady-state bioavailability of kiwifruit-derived versus synthetic vitamin C to plasma, semen, peripheral blood leukocytes, and skeletal muscle tissue [77]. Thus, other nutrients and phytochemicals present in kiwifruit are neither enhancing nor inhibiting the uptake of vitamin C from the whole fruit in humans.

4. Pharmacokinetic Bioavailability Studies in Humans

Pharmacokinetic studies show transient changes in plasma vitamin C levels and urinary excretion over the hours following ingestion of the vitamin C-containing test substance (relevant studies are shown in Table 3). Supplemental vitamin C typically takes about two hours to reach maximal plasma levels following ingestion. An early animal study found that vitamin C provided in citrus fruit media took longer to reach peak plasma concentrations compared with a synthetic vitamin C solution and also provided a larger area under the plasma vitamin C concentration-time curve [65]. These same investigators observed a comparable trend in human subjects supplemented with 500 mg vitamin C in the presence or absence of a citrus fruit extract [81]. The citrus fruit extract delayed maximal plasma levels by one hour and provided a 35% increase in vitamin C bioavailability. Interestingly, the citrus fruit extract increased 24 h urinary vitamin C excretion in participants pre-saturated with vitamin C, but decreased excretion in non-saturated participants compared with synthetic vitamin C alone. This suggests that the baseline vitamin C status of the individual may affect the comparative bioavailability of vitamin C. Although two other studies showed increased urinary excretion in vitamin C pre-saturated
subjects in the presence of fruit juice [71,82], another pre-saturation study showed comparable plasma
levels and 24 h urinary excretion in the presence of mixed bioflavonoids [83]. It should be noted that
doses of 500 mg vitamin C have reduced intestinal bioavailability [78] and are significantly higher
than would be obtained through a normal daily diet.

A number of pharmacokinetic studies have shown comparable bioavailability of vitamin C supplied
in synthetic form or in the presence of foods or fruit juices [84–88]. Nelson et al. [88] used
an intestinal triple lumen tube perfusion model to investigate the absorption of synthetic vitamin C and
that from an orange juice solution. This method allows direct measurement of intraluminal events
and showed no difference in the absorption of vitamin C from the two test solutions. A few
pharmacokinetic studies have shown transient decreases in plasma vitamin C levels and/or urinary
excretion at specific time points in the presence of food and fruit juices [34,71,84,85].
The physiological relevance of these transient differences is, however, likely minimal.

We recently carried out a pharmacokinetic bioavailability study of synthetic versus
kiwifruit-derived vitamin C in nine non-smoking males (aged 18–35 years) who had “healthy” or
“optimal” (i.e., >50 μmol/L) baseline levels of plasma vitamin C [89]. The participants received
either a chewable tablet (200 mg vitamin C) or the equivalent dose from gold kiwifruit. Fasting blood
and urine were collected half hourly to hourly over the eight hours following intervention. Plasma
ascorbate levels increased from 0.5 h post intervention, although no significant differences in the
plasma time-concentration curves were observed between the two interventions. An estimate of the
total increase in plasma ascorbate indicated complete uptake of the ingested vitamin C tablet and
kiwifruit-derived vitamin C. There was an increase in urinary ascorbate excretion, relative to urinary
creatinine, from two hours post intervention. There was also a significant difference between the two
interventions, with enhanced ascorbate excretion observed in the kiwifruit group. Urinary excretion
was calculated as ~40% and ~50% of the ingested dose from the vitamin C tablet and kiwifruit arms,
respectively. Overall, our pharmacokinetic study showed comparable relative bioavailability of
kiwifruit-derived vitamin C and synthetic vitamin C [89].
Table 1. Vitamin C comparative bioavailability studies in animal models.

| Animal Model | Intervention | Study Design | Vitamin C Analysis | Bioavailability Findings: Natural vs. Synthetic Vitamin C | Bioavailability Summary: Natural vs. Synthetic Vitamin C | Reference |
|--------------|--------------|--------------|--------------------|----------------------------------------------------------|----------------------------------------------------------|-----------|
| Gulo<sup>-/-</sup> mice | 0.5–5 mg/day vitamin C solution | 4 weeks intervention | HPLC-ECD | Kiwifruit ↑ serum, leukocyte, heart, liver, and kidney, but not brain vitamin C | Enhanced uptake in 5/6 pools | [68] |
| | Kiwifruit gel | | | | | |
| | | | | | | |
| CD rats | 60 mg/kg vitamin C gavage | Single dose; 4 h sampling | HPLC-ECD | Quercetin ↓ plasma vitamin C (at 4 h) | Decreased uptake in 1/1 pool | [54] |
| | 15 mg/kg quercetin | | | | | |
| | 50 mg vitamin C solution | Single dose; 4 h sampling | Fluorometric (NQSA) | Citrus fruit media ↑ plasma AUC | Enhanced uptake in 1/1 pool | [65] |
| | Citrus fruit media | | | | | |
| | | | | | | |
| | 1 mg/kg vitamin C (low vitamin C diet) | 26 days intervention | Colorimetric (DCPIP) | Orange peel extract ↑ adrenal, spleen and leukocyte, but not brain vitamin C; hesperidin ↑ adrenal and leukocyte, but not spleen vitamin C | Enhanced uptake in 3/4 pools | [61] |
| | | | | | | |
| | 50 mg/kg orange peel extract | | | | | |
| | 50 mg/kg hesperidin | | | | | |
| | 5 mg/kg vitamin C solution | 23 days intervention | Colorimetric (DCPIP) | Black current juice ↑ adrenal and spleen vitamin C; acerola cherry juice comparable adrenal and spleen vitamin C | Enhanced uptake in 2/2 organs | [64] |
| | Black current juice | | | | | |
| | Acerola cherry juice | | | | | |
| Guinea pigs | 0, 5 and 10 mg/kg vitamin C | 3 weeks intervention | Colorimetric (DNPH) | Rutin ↑ adrenal, but not liver vitamin C | Enhanced uptake in 1/2 pools | [62] |
| | 50 mg rutin | | | | | |
| | | | | | | |
| | 4 mg/kg vitamin C (low vitamin C diet) | 22 days intervention | Colorimetric (DNPH) | Rutin ↑ adrenal, but not liver or whole blood vitamin C of adequate animals | Enhanced uptake in 1/3 pools | [63] |
| | 10 mg rutin tablet | | | | | |
| | | | | | | |
| | 18 mg/kg vitamin C (adequate vitamin C diet) | | | | | |
| | 10 mg rutin tablet | | | | | |
| | | | | | | |
| | | | | | | |
| | Basic diet | 23 days intervention | Colorimetric (DCPIP) | Vitamin C + catechin ↑ liver, spleen, kidney, and adrenal vitamin C | Enhanced uptake in 4/4 organs | [42] |
| | | | | | | |
| | 1 mg/animal catechin | | | | | |
| | 10 mg/animal vitamin C | | | | | |
| | Vitamin C + catechin | | | | | |
| | 0.5 mg/day vitamin C solution | 20 days intervention | Colorimetric (DNPH) | Lemon juice comparable plasma and adrenal vitamin C | Comparable uptake in 2/2 pools | [67] |
| | 1 mL lemon juice | | | | | |

NQSA: 1,2-naphthoquinone-4-sulfonic acid; AUC: area under the concentration-time curve; DNPH: 2,4-dinitrophenylhydrazine; DCPIP: 2,6-dichlorophenolindophenol.
Table 2. Steady state comparative bioavailability studies in humans.

| Subjects | Intervention | Study Design | Vitamin C Analysis | Bioavailability Findings: Natural vs. Synthetic Vitamin C | Bioavailability Summary: Natural vs. Synthetic Vitamin C | Reference |
|----------|--------------|--------------|--------------------|----------------------------------------------------------|-------------------------------------------------------|-----------|
| 36 non-smoking males 18–35 years | 50 mg/day vitamin C tablet Gold kiwifruit (50 mg vitamin C) | 6 weeks of supplementation; Parallel design | HPLC-ECD | Kiwifruit comparable plasma, urine, semen, mononuclear cell, neutrophil and muscle tissue vitamin C | Comparable uptake in 6/6 pools | [77] |
| 11 non-smoking women 21–39 years | 69 mg/day vitamin C capsule Orange juice (66 mg vitamin C) | 2 weeks of supplementation; Crossover design (2 week washout) | Colorimetric (DNPH) | Orange juice comparable plasma vitamin C | Comparable uptake in 1/1 pool | [76] |
| 68 non-smoking males 30–59 years | 108 mg/day vitamin C tablet Orange—pieces or juice Broccoli—cooked or raw | 4 weeks of supplementation; Crossover design (4 week washout) | Colorimetric (DNPH) | Orange pieces/juice or cooked broccoli comparable plasma vitamin C; raw broccoli ↓ plasma vitamin C | Comparable uptake in 1/1 pool | [35] |
| 14 men and women | 75 mg/day vitamin C Papayas (75 mg/day vitamin C) Guava juice (75 mg/day vitamin C) | Sequential design | Colorimetric | Papayas and guava juice comparable plasma and urinary vitamin C | Comparable uptake in 2/2 pools | [72] |
| 4 healthy young subjects | 75 mg/day vitamin C tablets Raw cabbage (75 mg/day vitamin C) Tomato juice (75 mg/day vitamin C) | Pre-study saturation; Sequential design | Colorimetric | Raw cabbage and tomato juice comparable plasma and urinary vitamin C | Comparable uptake in 2/2 pools | [73] |
| 7 college women | 40 mg/day vitamin C solution Red raspberries (40 mg/day vitamin C) | Pre-study saturation; Sequential design | Colorimetric (DCPIP) | Raspberries comparable blood and urinary vitamin C | Comparable uptake in 2/2 pools | [74] |
| 12 young adults | 100 mg/day vitamin C Orange juice (100 mg/day vitamin C) | Sequential design | Colorimetric (DCPIP) | Orange juice comparable urinary vitamin C | Comparable uptake in 1/1 pool | [75] |

DNPH: 2,4-dinitrophenylhydrazine; DCPIP: 2,6-dichlorophenolindophenol.
### Table 3. Pharmacokinetic comparative bioavailability studies in humans.

| Subjects                        | Intervention                          | Study Design                      | Vitamin C Analysis | Plasma Uptake                                                                 | Urinary Excretion                                                                 | Reference |
|---------------------------------|---------------------------------------|-----------------------------------|-------------------|-------------------------------------------------------------------------------|--------------------------------------------------------------------------------|-----------|
| 9 non-smoking males 18–35 years | 200 mg vitamin C tablet Gold kiwifruit (200 mg vitamin C) | 8 h sampling; Crossover design (3 week washout) | HPLC-ECD          | Kiwifruit comparable plasma vitamin C and AUC                                  | Kiwifruit ↑ urinary vitamin C and AUC (relative to creatinine)                   | [89]      |
| 5 non-smoking males 22–27 years | 50 mg vitamin C solution 282 g mashed potato (50 mg vitamin C) 87 g potato chips (50 mg vitamin C) Placebo | 8 h sampling; Crossover design (4 week washout) | HPLC-ECD          | Mashed potatoes ↓ plasma vitamin C (at 1 to 2.5 h); potato chips ↓ AUC          | Mashed potatoes ↓ urinary vitamin C (at 3 h)                                     | [84]      |
| 5 non-smoking males 22–26 years | 50–500 mg vitamin C solution 100 mL acerola juice (50 mg vitamin C) | 6 h sampling; Crossover design | HPLC-ECD          | Acerola juice comparable plasma vitamin C and AUC                             | Acerola juice ↓ urinary vitamin C (at 1, 2 and 5 h)                             | [85]      |
| 12 males 20–35 years            | 284 mg vitamin C drink 590 mL orange juice (68 mg vitamin C) Placebo (milk) | 4.5 h sampling; Crossover design (1 week washout) | Colorimetric (TPTZ) | Orange juice comparable bioavailability (AUC/concentration)                       | ND                                                | [86]      |
| 7 non-smoking females            | 150 mg vitamin C solution 300 mL orange juice (150 mg vitamin C) Placebo | 8 h sampling; Crossover design (2 week washout) | HPLC-UV           | Orange juice comparable plasma vitamin C                                      | ND                                                | [87]      |
| 7 non-smokers 26–59 years       | 30 mg vitamin C solution 200 mL red grape juice (30 mg vitamin C) Placebo | 4 h sampling; Crossover design (3–4 week washout) | Fluorometric (phenylene diamine) | Grape juice ↓ plasma vitamin C (at 16 to 28 min)                               | ND                                                | [34]      |
| 9 healthy subjects 19–41 years   | 500 mg vitamin C tablet Mixed bioflavonoids Placebo | 1 g/day vitamin C for 2 weeks pre-study; 8 h sampling; Crossover design (1 week washout) | Colorimetric (DNPH) | Bioflavonoids comparable AUC                                                   | Bioflavonoids comparable 24 h vitamin C excretion                                 | [83]      |
| 12 non-smoking subjects 18–41 years | 500 mg vitamin C solution 2 g citrus extract Placebo | Subgroup had 1 g/day vitamin C for 2 weeks pre-study; 8 h sampling; Crossover design (1 week washout) | Fluorometric      | Citrus extract ↑ AUC                                                          | Citrus extract ↓ 24 h vitamin C excretion in non-saturated subjects and ↑ 24 h vitamin C excretion in saturated subjects | [81]      |
### Table 3. Cont.

| 5 men  | 500 mg vitamin C solution | 100 mg/day vitamin C for 1 month pre-study; 8 h sampling; Crossover design (1 week washout) | Colorimetric (Indophenol dye) | ND | Blackcurrant juice slight ↑ 8 h vitamin C excretion in saturated subjects |
|--------|---------------------------|---------------------------------------------------------------------------------------------|-------------------------------|----|--------------------------------------------------------------------------------|
| 21–25 years | 500 mg vitamin C in blackcurrant juice |                                                                                  |                               |    |                                                                                  |

| 15 normal subjects (4 smokers) | 70 mg/h vitamin C solution | Intestinal perfusion; Tandem design | Colorimetric (DNPH) | Orange juice comparable intestinal absorption | ND | Orange juice slight ↑ 8 h vitamin C excretion in saturated subjects |
| 20–42 years | Orange juice |                                      |                               |                                      |    |                                                                                  |

| 12 men (6 smokers) | 75 mg vitamin C solution 400 mg rutin | Pre- and post-saturation with 1 mg/day vitamin C; 2–24 h sampling; Crossover design (1 day washout) | Colorimetric | Orange juice and rutin ↓ plasma vitamin C (at 2 h) | Orange juice slight ↑ 24 h vitamin C excretion |
| 23–44 years | Orange juice (75 mg vitamin C) | Crossover design (1 day washout) |                               |                                      |    |                                                                                  |

AUC: area under the concentration-time curve; ND: not determined; DNPH: 2,4-dinitrophenylhydrazine; TPTZ: 2,4,6-tris(2-pyridyl)-s-triazine.
5. Vitamin C Bioavailability from Different Tablet Formulations

Doses of vitamin C up to 2000 mg/day are considered safe for general consumption [90]. However, pharmacokinetic studies indicate that ingestion of single doses of vitamin C greater than 200 mg have lower relative bioavailability [78], indicating that ingestion of several smaller doses each day is preferable to a single large dose. A number of studies have investigated the relative bioavailability of vitamin C from different tablet formulations and have shown that slow-release formulations provide superior vitamin bioavailability [91–94]. Salts of vitamin C, such as sodium and calcium ascorbate (Ester-C), have also been tested. Animal studies indicated that Ester-C (which contains calcium ascorbate, as well as DHA and calcium threonate) was absorbed more readily and excreted less rapidly than ascorbic acid [95] and had superior anti-scorbutic activity in ODS rats [96]. Johnston and Luo [83], however, found no significant differences between Ester-C and ascorbic acid bioavailability in humans. Nevertheless, Ester-C has been shown to be better tolerated in individuals sensitive to acidic foods [97].

6. Conclusions

Overall, a majority of animal studies have shown differences in the comparative bioavailability of synthetic versus food-derived vitamin C, or vitamin C in the presence of isolated bioflavonoids, although the results varied depending on the animal model, study design and body compartments measured. In contrast, all steady state comparative bioavailability studies in humans have shown no differences between synthetic and natural vitamin C, regardless of the subject population, study design or intervention used. Some pharmacokinetic studies in humans have shown transient and small comparative differences between synthetic and natural vitamin C, although these differences are likely to have minimal physiological impact. Thus, not only do the reviewed studies reiterate the injunction that the findings of animal studies should not be directly translated to humans [98,99], but it is also apparent that additional comparative bioavailability studies in humans are unwarranted.

Although synthetic and food-derived vitamin C appear to be equally bioavailable in humans, ingesting vitamin C as part of a whole food is considered preferable because of the concomitant consumption of numerous other macro- and micronutrients and phytochemicals, which will confer additional health benefits. Numerous epidemiological studies have indicated that higher intakes of fruit and vegetables are associated with decreased incidence of stroke [100], coronary heart disease [101], and cancers at various sites [102,103]. Vitamin C status is one of the best markers for fruit and vegetable intake [104], and food-derived vitamin C is associated with decreased incidence of numerous chronic diseases [1], however, whether the observed health effects of fruit and vegetable ingestion are due to vitamin C and/or other plant-derived components is currently unknown. With respect to coronary heart disease, strong evidence exists for a protective effect of vegetables, moderate evidence for fruit and dietary vitamin C and insufficient evidence for supplemental vitamin C [105]. Some meta-analyses support the premise that dietary vitamin C is more protective than supplements [106], while others show reduced disease incidence with supplemental but not dietary vitamin C [107].
A major limitation with epidemiological studies is that they show only an association between dietary vitamin C intake and disease risk and cannot ascertain whether different sources of vitamin C (i.e., food-derived versus supplement) are a cause, consequence, or simply a correlate of the particular end-point measured. Interpretations can also vary significantly depending on the input of different confounders [108]. Furthermore, epidemiological studies rely predominantly on food frequency questionnaires [109,110] and 24 h dietary recalls [111] to ascertain vitamin C intakes from foods and/or supplements [112]. This methodology has numerous limitations [113] and correlations with vitamin C status can vary depending on the methods employed as well as numerous other external factors [114]. Pooled or meta-analyses of epidemiological studies are particularly problematic due to the combining of variable study designs, cohorts and endpoints, often resulting in dilution or misinterpretation of study findings.

The gold standard for determining causality is the double-blind randomized placebo controlled clinical trial. Although this type of study design works well for comparing the effects of drugs against a placebo, it does not work for nutrients, such as vitamin C, which are already in the food chain and are required for life, i.e., there is no true placebo. Numerous other methodological issues have been identified with the design of many clinical trials investigating the health effects of vitamin C [115]. For example, a major flaw with many vitamin C intervention studies is the use of study populations with already adequate or even saturating vitamin C levels, which significantly decreases the likelihood of observing any effects of the intervention. Thus, it is recommended that study populations are comprised of individuals with sub-optimal vitamin C status (i.e., <50 μmol/L plasma vitamin C) or that sub-group analysis is carried out on the low vitamin C sub-populations [116]. With pharmacokinetic studies, both unsaturated and saturated individuals can be used, but comparative bioavailability studies have shown that results may vary depending on the baseline vitamin C status of the study subjects. Furthermore, the vitamin C doses chosen for intervention are critical since doses above 200 mg have decreased intestinal uptake [78], indicating that if higher doses are warranted then these should be provided as multiple doses of ~200 mg each to ensure complete bioavailability.

The comparative health effects of supplemental versus food-derived vitamin C will only be determined through the use of appropriate and well-designed studies. Determination of the physiological effects or health outcomes of intervention with synthetic versus natural vitamin C will depend largely on the endpoints measured. Only a handful of comparative intervention studies have been carried out to assess specific physiological or health endpoints. Guarnieri et al. [89] investigated potential protection of mononuclear leukocytes from supplemented individuals against ex vivo oxidative DNA damage. Although they found comparable vitamin C bioavailability between a single portion of orange juice (containing 150 mg vitamin C) and a synthetic vitamin C drink of the same dosage, they showed that only the orange juice protected the leukocytes from ex vivo oxidative DNA damage [89]. However, how closely ex vivo oxidation of DNA resembles events occurring in vivo is debatable and results could also vary significantly depending on the type of oxidative stress. Johnston et al. [76] compared plasma lipid peroxidation in individuals who had been supplemented with either orange juice or synthetic vitamin C (~70 mg/day) for two weeks. They found comparable vitamin C bioavailability and a similar reduction in lipid peroxidation with both interventions [76]. Several studies have assessed the effects of synthetic and natural vitamin C, or vitamin C in the presence of bioflavonoids, on the common cold. Two earlier studies showed a lack of an effect of
vitamin C (~200 mg/day), with and without purified bioflavonoids, on the prevention and cure of the common cold [117,118]. Another study indicated that synthetic vitamin C (80 mg/day) and orange juice both decreased the symptoms of the common cold compared with placebo, but there were no differences between the two interventions [119].

As alluded to in the introduction, vitamin C is known to enhance the bioavailability of other nutrients, such as vitamin E [30] and non-heme iron [31,32], which may enhance the health effects of vitamin C-containing foods. Bioflavonoids are also known to have numerous biological activities [120]. Recently vitamin C has been shown to modulate specific biological activities of quercetin and tea polyphenols [121,122]. Thus, future studies may elucidate the physiological relevance of these interactions.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

Co-funding was provided by the New Zealand Ministry of Business, Innovation & Employment and Zespri International Ltd., Mount Maunganui, New Zealand.

References

1. Carr, A.C.; Frei, B. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *Am. J. Clin. Nutr.* 1999, 69, 1086–1107.
2. Englard, S.; Seifter, S. The biochemical functions of ascorbic acid. *Annu. Rev. Nutr.* 1986, 6, 365–406.
3. Ozer, A.; Bruick, R.K. Non-heme dioxygenases: Cellular sensors and regulators jelly rolled into one? *Nat. Chem. Biol.* 2007, 3, 144–153.
4. Monfort, A.; Wutz, A. Breathing-in epigenetic change with vitamin C. *EMBO Rep.* 2013, 14, 337–346.
5. Hornig, D. Distribution of ascorbic acid, metabolites and analogues in man and animals. *Ann. N. Y. Acad. Sci.* 1975, 258, 103–118.
6. Omaye, S.T.; Schaus, E.E.; Kutnink, M.A.; Hawkes, W.C. Measurement of vitamin C in blood components by high-performance liquid chromatography. Implication in assessing vitamin C status. *Ann. N. Y. Acad. Sci.* 1987, 498, 389–401.
7. Tsao, C.S. An Overview of Ascorbic Acid Chemistry and Biochemistry. In *Vitamin C in Health and Disease*; Packer, L., Fuchs, J., Eds.; Marcel Dekker: New York, NY, USA, 1997; pp. 25–58.
8. Nishikimi, M.; Fukuyama, R.; Minoshima, S.; Shimizu, N.; Yagi, K. Cloning and chromosomal mapping of the human nonfunctional gene for L-gulono-γ-lactone oxidase, the enzyme for L-ascorbic acid biosynthesis missing in man. *J. Biol. Chem.* 1994, 269, 13685–13688.
9. Krebs, H.A. The Sheffield Experiment on the vitamin C requirement of human adults. *Proc. Nutr. Soc.* 1953, 12, 237–246.
10. Sauberlich, H.E. A History of Scurvy and Vitamin C. In: Vitamin C in Health and Disease; Packer, L., Fuchs, J., Eds.; Marcel Dekker: New York, NY, USA, 1997; pp. 1–24.

11. Michels, A.J.; Hagen, T.M.; Frei, B. Human genetic variation influences vitamin C homeostasis by altering vitamin C transport and antioxidant enzyme function. Annu. Rev. Nutr. 2013, 33, 45–70.

12. Tsukaguchi, H.; Tokui, T.; Mackenzie, B.; Berger, U.V.; Chen, X.Z.; Wang, Y.; Brubaker, R.F.; Hediger, M.A. A family of mammalian Na⁺-dependent L-ascorbic acid transporters. Nature 1999, 399, 70–75.

13. Savini, I.; Rossi, A.; Pierro, C.; Avigliano, L.; Catani, M.V. SVCT1 and SVCT2: Key proteins for vitamin C uptake. Amino Acids 2008, 34, 347–355.

14. Rumsey, S.C.; Welch, R.W.; Garraffo, H.M.; Ge, P.; Lu, S.F.; Crossman, A.T.; Kirk, K.L.; Levine, M. Specificity of ascorbic acid analogs for ascorbate transport. Synthesis and detection of [(125)I]6-deoxy-6-iodo-L-ascorbic acid and characterization of its ascorbate-specific transport properties. J. Biol. Chem. 1999, 274, 23215–23222.

15. Goldman, H.M.; Gould, B.S.; Munro, H.N. The antiscorbutic action of L-ascorbic acid and d-isoascorbic acid (erythorbic acid) in the guinea pig. Am. J. Clin. Nutr. 1981, 34, 24–33.

16. Hughes, R.E.; Hurley, R.J. The uptake of d-araboascorbic acid (d-isoascorbic acid) by guinea-pig tissues. Br. J. Nutr. 1969, 23, 211–216.

17. Levine, M. Fruits and vegetables: There is no substitute. Am. J. Clin. Nutr. 1996, 64, 381–382.

18. Sauberlich, H.E.; Tamura, T.; Craig, C.B.; Freeberg, L.E.; Liu, T. Effects of erythorbic acid on vitamin C metabolism in young women. Am. J. Clin. Nutr. 1996, 64, 336–346.

19. Corpe, C.P.; Eck, P.; Wang, J.; Al-Hasani, H.; Levine, M. Intestinal dehydroascorbic acid (DHA) transport mediated by the facilitative sugar transporters, GLUT2 and GLUT8. J. Biol. Chem. 2013, 288, 9092–9101.

20. Rumsey, S.C.; Kwon, O.; Xu, G.W.; Burant, C.F.; Simpson, I.; Levine, M. Glucose transporter isoforms GLUT1 and GLUT3 transport dehydroascorbic acid. J. Biol. Chem. 1997, 272, 18982–18989.

21. Vera, J.C.; Rivas, C.I.; Fischbarg, J.; Golde, D.W. Mammalian facilitative hexose transporters mediate the transport of dehydroascorbic acid. Nature 1993, 364, 79–82.

22. Washko, P.W.; Wang, Y.; Levine, M. Ascorbic acid recycling in human neutrophils. J. Biol. Chem. 1993, 268, 15531–15535.

23. Corti, A.; Casini, A.F.; Pompella, A. Cellular pathways for transport and efflux of ascorbate and dehydroascorbate. Arch. Biochem. Biophys. 2010, 500, 107–115.

24. Nishiyama, I.; Yamashita, Y.; Yamanaka, M.; Shimohashi, A.; Fukuda, T.; Oota, T. Varietal difference in vitamin C content in the fruit of kiwifruit and other actinidia species. J. Agric. Food Chem. 2004, 52, 5472–5475.

25. Dhariwal, K.R.; Hartzell, W.O.; Levine, M. Ascorbic acid and dehydroascorbic acid measurements in human plasma and serum. Am. J. Clin. Nutr. 1991, 54, 712–716.

26. Ogiri, Y.; Sun, F.; Hayami, S.; Fujimura, A.; Yamamoto, K.; Yaita, M.; Kojo, S. Very low vitamin C activity of orally administered L-dehydroascorbic acid. J. Agric. Food Chem. 2002, 50, 227–229.
27. Welch, R.W.; Wang, Y.; Crossman, A., Jr.; Park, J.B.; Kirk, K.L.; Levine, M. Accumulation of vitamin C (ascorbate) and its oxidized metabolite dehydroascorbic acid occurs by separate mechanisms. *J. Biol. Chem.* 1995, 270, 12584–12592.

28. Wang, Y.; Russo, T.A.; Kwon, O.; Chanock, S.; Runsey, S.C.; Levine, M. Ascorbate recycling in human neutrophils: Induction by bacteria. *Proc. Natl. Acad. Sci. USA* 1997, 94, 13816–13819.

29. Packer, J.E.; Slater, T.F.; Willson, R.L. Direct observation of a free radical interaction between vitamin E and vitamin C. *Nature* 1979, 278, 737–738.

30. Tanaka, K.; Hashimoto, T.; Tokumaru, S.; Iguchi, H.; Kojo, S. Interactions between vitamin C and vitamin E are observed in tissues of inherently scorbutic rats. *J. Nutr.* 1997, 127, 2060–2064.

31. Beck, K.; Conlon, C.A.; Kruger, R.; Coad, J.; Stonehouse, W. Gold kiwifruit consumed with an iron-fortified breakfast cereal meal improves iron status in women with low iron stores: A 16-week randomised controlled trial. *Br. J. Nutr.* 2011, 105, 101–109.

32. Hallberg, L.; Brune, M.; Rossander, L. Effect of ascorbic acid on iron absorption from different types of meals. Studies with ascorbic-acid-rich foods and synthetic ascorbic acid given in different amounts with different meals. *Hum. Nutr. Appl. Nutr.* 1986, 40, 97–113.

33. Scheers, N.M.; Sandberg, A.S. Iron regulates the uptake of ascorbic acid and the expression of sodium-dependent vitamin C transporter 1 (SVCT1) in human intestinal Caco-2 cells. *Br. J. Nutr.* 2011, 105, 1734–1740.

34. Bates, C.J.; Jones, K.S.; Bluck, L.J. Stable isotope-labelled vitamin C as a probe for vitamin C absorption by human subjects. *Br. J. Nutr.* 2004, 91, 699–705.

35. Mangels, A.R.; Block, G.; Frey, C.M.; Patterson, B.H.; Taylor, P.R.; Norkus, E.P.; Levander, O.A. The bioavailability to humans of ascorbic acid from oranges, orange juice and cooked broccoli is similar to that of synthetic ascorbic acid. *J. Nutr.* 1993, 123, 1054–1061.

36. Keltz, F.R.; Kies, C.; Fox, H.M. Urinary ascorbic acid excretion in the human as affected by dietary fiber and zinc. *Am. J. Clin. Nutr.* 1978, 31, 1167–1171.

37. Rusznyak, S.; Szent-Gyorgyi, A. Vitamin P: Flavonols as vitamins. *Nature* 1936, 138, 27.

38. Bentsath, A.; Rusznyak, S.; Szent-Gyorgyi, A. Vitamin nature of flavones. *Nature* 1936, 138, 798.

39. Bentsath, A.; Rusznyak, S.; Szent-Gyorgyi, A. Vitamin P. *Biochem. J.* 1937, 31, 915–919.

40. Cotereau, H.; Gabe, M.; Géoro, E.; Parrot, J.-L. Influence of vitamin P (vitamin C₂) upon the amount of ascorbic acid in the organs of the guinea pig. *Nature* 1948, 161, 557.

41. Scarbrough, H. Deficiency of vitamin C and vitamin P in man. *Lancet* 1940, 236, 644–647.

42. Scarbrough, H. Vitamin P. *Biochem. J.* 1939, 33, 1400–1407.

43. Elmby, A.; Warburg, E. The inadequacy of synthetic ascorbic acid as an antiscorbutic agent. *Lancet* 1937, 230, 1363–1365.

44. Ivanov, V.; Carr, A.C.; Frei, B. Red wine antioxidants bind to human lipoproteins and protect them from metal ion-dependent and -independent oxidation. *J. Agric. Food Chem.* 2001, 49, 4442–4449.

45. Bors, W.; Michel, C.; Saran, M. Flavonoid antioxidants: rate constants for reactions with oxygen radicals. *Methods Enzymol.* 1994, 234, 420–429.
48. Beker, B.Y.; Sonmezoglu, I.; Imer, F.; Apak, R. Protection of ascorbic acid from copper(II)-catalyzed oxidative degradation in the presence of flavonoids: Quercetin, catechin and morin. *Int. J. Food Sci. Nutr.* **2011**, *62*, 504–512.

49. Clemetson, C.A.; Andersen, L. Plant polyphenols as antioxidants for ascorbic acid. *Ann. N. Y. Acad Sci.* **1966**, *136*, 341–376.

50. Harper, K.A.; Morton, A.D.; Rolfe, E.J. The phenolic compounds of blackcurrant juice and their protective effect on ascorbic acid III. The mechanism of ascorbic acid oxidation and its inhibition by flavonoids. *Int. J. Food Sci. Technol.* **1969**, *4*, 255–267.

51. Clegg, K.M.; Morton, A.D. The phenolic compounds of blackcurrant juice and their protective effect on ascorbic acid II. The stability of ascorbic acid in model systems containing some of the phenolic compounds associated with blackcurrant juice. *Int. J. Food Sci. Technol.* **1968**, *3*, 277–284.

52. Carr, A.; Frei, B. Does vitamin C act as a pro-oxidant under physiological conditions? *FASEB J.* **1999**, *13*, 1007–1024.

53. Lotito, S.B.; Frei, B. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: Cause, consequence, or epiphenomenon? *Free Radic. Biol. Med.* **2006**, *41*, 1727–1746.

54. Song, J.; Kwon, O.; Chen, S.; Daruwala, R.; Eck, P.; Park, J.B.; Levine, M. Flavonoid inhibition of sodium-dependent vitamin C transporter 1 (SVCT1) and glucose transporter isoform 2 (GLUT2), intestinal transporters for vitamin C and Glucose. *J. Biol. Chem.* **2002**, *277*, 15252–15260.

55. Park, J.B.; Levine, M. Intracellular accumulation of ascorbic acid is inhibited by flavonoids via blocking of dehydroascorbic acid and ascorbic acid uptakes in HL-60, U937 and Jurkat cells. *J. Nutr.* **2000**, *130*, 1297–1302.

56. Corpe, C.P.; Lee, J.H.; Kwon, O.; Eck, P.; Narayanan, J.; Kirk, K.L.; Levine, M. 6-Bromo-6-deoxy-L-ascorbic acid: An ascorbate analog specific for Na\(^+\)-dependent vitamin C transporter but not glucose transporter pathways. *J. Biol. Chem.* **2005**, *280*, 5211–5220.

57. Washko, P.W.; Welch, R.W.; Dhariwal, K.R.; Wang, Y.; Levine, M. Ascorbic acid and dehydroascorbic acid analyses in biological samples. *Anal. Biochem.* **1992**, *204*, 1–14.

58. Mizushima, Y.; Harauchi, T.; Yoshizaki, T.; Makino, S. A rat mutant unable to synthesize vitamin C. *Experientia* **1984**, *40*, 359–361.

59. Maeda, N.; Hagihara, H.; Nakata, Y.; Hiller, S.; Wilder, J.; Reddick, R. Aortic wall damage in mice unable to synthesize ascorbic acid. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 841–846.

60. Mohan, S.; Kapoor, A.; Singgih, A.; Zhang, Z.; Taylor, T.; Yu, H.; Chadwick, R.B.; Chung, Y.S.; Donahue, L.R.; Rosen, C.; *et al.* Spontaneous fractures in the mouse mutant *sfx* are caused by deletion of the gulonolactone oxidase gene, causing vitamin C deficiency. *J. Bone Miner. Res.* **2005**, *20*, 1597–1610.

61. Wilson, H.K.; Price-Jones, C.; Hughes, R.E. The influence of an extract of orange peel on the growth and ascorbic acid metabolism of young guinea-pigs. *J. Sci. Food Agric.* **1976**, *27*, 661–666.

62. Douglass, C.D.; Kamp, G.H. The effect of orally administered rutin on the adrenal ascorbic acid level in guinea pigs. *J. Nutr.* **1959**, *67*, 531–536.
63. Papageorge, E.; Mitchell, G.L., Jr. The effect of oral administration of rutin on blood, liver and adrenal ascorbic acid and on liver and adrenal cholesterol in guinea pigs. *J. Nutr.* 1949, 37, 531–540.

64. Hughes, R.E.; Hurley, R.J.; Jones, P.R. The retention of ascorbic acid by guinea-pig tissues. *Br. J. Nutr.* 1971, 26, 433–438.

65. Vinson, J.A.; Bose, P. Comparative bioavailability of synthetic and natural vitamin C in guinea pigs. *Nutr. Rep. Int.* 1983, 27, 875–879.

66. Ambrose, A.M.; De, E.F. The value of rutin and quercetin in scurvy. *J. Nutr.* 1949, 38, 305–317.

67. Todhunter, E.N.; Robbins, R.C.; Ivey, G.; Brewer, W. A comparison of the utilization by guinea pigs of equivalent amounts of ascorbic acid (vitamin C) in lemon juice and in crystalline form. *J. Nutr.* 1940, 19, 113–120.

68. Vissers, M.C.M.; Bozonet, S.M.; Pearson, J.F.; Braithwaite, L.J. Dietary ascorbate affects steady state tissue levels in vitamin C-deficient mice: Tissue deficiency after sub-optimal intake and superior bioavailability from a food source (kiwifruit). *Am. J. Clin. Nutr.* 2011, 93, 292–301.

69. Latocha, P.; Krupa, T.; Wolosiak, R.; Worobiej, E.; Wilczak, J. Antioxidant activity and chemical difference in fruit of different *Actinidia* sp. *Int J. Food Sci. Nutr.* 2010, 61, 381–394.

70. Fiorentino, A.; D’Abrosca, B.; Pacifico, S.; Mastellone, C.; Scognamiglio, M.; Monaco, P. Identification and assessment of antioxidant capacity of phytochemicals from kiwi fruits. *J. Agric. Food Chem.* 2009, 57, 4148–4155.

71. Pelletier, O.; Keith, M.O. Bioavailability of synthetic and natural ascorbic acid. *J. Am. Diet. Assoc.* 1974, 64, 271–275.

72. Hartzler, E.R. The availability of ascorbic acid in papayas and guavas. *J. Nutr.* 1945, 30, 355–365.

73. Clayton, M.M.; Borden, R.A. The availability for human nutrition of the vitamin C in raw cabbage and home-canned tomato juice. *J. Nutr.* 1943, 25, 349–369.

74. Todhunter, E.N.; Fatzer, A.S. A comparison of the utilization by college women of equivalent amounts of ascorbic acid (vitamin C) in red raspberries and in crystalline form. *J. Nutr.* 1940, 19, 121–130.

75. Hawley, E.E.; Stephens, D.J.; Anderson, G. The excretion of vitamin C in normal individuals following a comparable quantitative administration in the form of orange juice, cevitamic acid by mouth and cevitamic acid intravenously. *J. Nutr.* 1936, 11, 135–145.

76. Johnston, C.S.; Dancho, C.L.; Strong, G.M. Orange juice ingestion and supplemental vitamin C are equally effective at reducing plasma lipid peroxidation in healthy adult women. *J. Am. Coll. Nutr.* 2003, 22, 519–523.

77. Carr, A.C.; Bozonet, S.M.; Pullar, J.M.; Simcock, J.W.; Vissers, M.C. A randomised steady-state bioavailability study of synthetic and natural (kiwifruit-derived) vitamin C. *Nutrients* 2013, 5, 3684–3695.

78. Levine, M.; Conry-Cantilena, C.; Wang, Y.; Welch, R.W.; Washko, P.W.; Dhariwal, K.R.; Park, J.B.; Lazarev, A.; Graumlich, J.F.; King, J.; et al. Vitamin C pharmacokinetics in healthy volunteers: Evidence for a recommended dietary allowance. *Proc. Natl. Acad. Sci. USA* 1996, 93, 3704–3709.
79. Block, G.; Mangels, A.R.; Patterson, B.H.; Levander, O.A.; Norkus, E.P.; Taylor, P.R. Body weight and prior depletion affect plasma ascorbate levels attained on identical vitamin C intake: A controlled-diet study. *J. Am. Coll. Nutr.* 1999, 18, 628–637.

80. Carr, A.C.; Bozonet, S.M.; Pullar, J.M.; Simcock, J.W.; Vissers, M.C. Human skeletal muscle ascorbate is highly responsive to changes in vitamin C intake and plasma concentrations. *Am. J. Clin. Nutr.* 2013, 97, 800–807.

81. Vinson, J.A.; Bose, P. Comparative bioavailability to humans of ascorbic acid alone or in a citrus extract. *Am. J. Clin. Nutr.* 1988, 48, 601–604.

82. Jones, E.; Hughes, R.E. The influence of bioflavonoids on the absorption of vitamin C. *IRCS J. Med. Sci.* 1984, 12, 320.

83. Johnston, C.S.; Luo, B. Comparison of the absorption and excretion of three commercially available sources of vitamin C. *J. Am. Diet. Assoc.* 1994, 94, 779–781.

84. Kondo, Y.; Higashi, C.; Iwama, M.; Ishihara, K.; Handa, S.; Mugita, H.; Maruyama, N.; Koga, H.; Ishigami, A. Bioavailability of vitamin C from mashed potatoes and potato chips after oral administration in healthy Japanese men. *Br. J. Nutr.* 2012, 107, 885–892.

85. Uchida, E.; Kondo, Y.; Amano, A.; Aizawa, S.; Hanamura, T.; Aoki, H.; Nagamine, K.; Koizumi, T.; Maruyama, N.; Ishigami, A. Absorption and excretion of ascorbic acid alone and in acerola (*Malpighia emarginata*) juice: Comparison in healthy japanese subjects. *Biol. Pharm. Bull.* 2011, 34, 1744–1747.

86. Carter, B.; Monsivais, P.; Drewnowski, A. Absorption of folic acid and ascorbic acid from nutrient comparable beverages. *J. Food Sci.* 2010, 75, H289–H293.

87. Guarnieri, S.; Riso, P.; Porrini, M. Orange juice vs vitamin C: Effect on hydrogen peroxide-induced DNA damage in mononuclear blood cells. *Br. J. Nutr.* 2007, 97, 639–643.

88. Nelson, E.W.; Streiff, R.R.; Cerda, J.J. Comparative bioavailability of folate and vitamin C from a synthetic and a natural source. *Am. J. Clin. Nutr.* 1975, 28, 1014–1019.

89. Carr, A.C.; Bozonet, S.M.; Vissers, M.C.M. A randomised cross-over pharmacokinetic bioavailability study of synthetic versus kiwifruit-derived vitamin C. *Nutrients* 2013, 5, 3684–3695.

90. Hathcock, J.N.; Azzi, A.; Blumberg, J.; Bray, T.; Dickinson, A.; Frei, B.; Jialal, I.; Johnston, C.S.; Kelly, F.J.; Kraemer, K.; et al. Vitamins E and C are safe across a broad range of intakes. *Am. J. Clin. Nutr.* 2005, 81, 736–745.

91. Yung, S.; Mayersohn, M.; Robinson, J.B. Ascorbic acid absorption in humans: A comparison among several dosage forms. *J. Pharm. Sci.* 1982, 71, 282–285.

92. Bhagavan, H.N.; Wolkoff, B.I. Correlation between the disintegration time and the bioavailability of vitamin C tablets. *Pharm. Res.* 1993, 10, 239–242.

93. Sacharin, R.; Taylor, T.; Chasseaud, L.F. Blood levels and bioavailability of ascorbic acid after administration of a sustained-release formulation to humans. *Int. J. Vitam. Nutr. Res.* 1977, 47, 68–74.

94. Nyyssonen, K.; Poulsen, H.E.; Hayn, M.; Agerbo, P.; Porkkala-Sarataho, E.; Kaikkonen, J.; Salonen, R.; Salonen, J.T. Effect of supplementation of smoking men with plain or slow release ascorbic acid on lipoprotein oxidation. *Eur. J. Clin. Nutr.* 1997, 51, 154–163.

95. Bush, M.J.; Verlangieri, A.J. An acute study on the relative gastro-intestinal absorption of a novel form of calcium ascorbate. *Res. Commun. Chem. Pathol. Pharmacol.* 1987, 57, 137–140.
96. Verlangieri, A.J.; Fay, M.J.; Bannon, A.W. Comparison of the anti-scorbutic activity of L-ascorbic acid and Ester C in the non-ascorbate synthesizing Osteogenic Disorder Shionogi (ODS) rat. Life Sci. 1991, 48, 2275–2281.

97. Gruenwald, J.; Graubaum, H.J.; Busch, R.; Bentley, C. Safety and tolerance of ester-C compared with regular ascorbic acid. Adv. Ther. 2006, 23, 171–178.

98. Knight, A. Systematic reviews of animal experiments demonstrate poor human clinical and toxicological utility. Altern. Lab. Anim. 2007, 35, 641–659.

99. Seok, J.; Warren, H.S.; Cuenca, A.G.; Mindrinos, M.N.; Baker, H.V.; Xu, W.; Richards, D.R.; McDonald-Smith, G.P.; Gao, H.; Hennessy, L.; et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc. Natl. Acad. Sci. USA 2013, 110, 3507–3512.

100. He, F.J.; Nowson, C.A.; MacGregor, G.A. Fruit and vegetable consumption and stroke: Meta-analysis of cohort studies. Lancet 2006, 367, 320–326.

101. Dauchet, L.; Amouyel, P.; Hercberg, S.; Dallongeville, J. Fruit and vegetable consumption and risk of coronary heart disease: A meta-analysis of cohort studies. J. Nutr. 2006, 136, 2588–2593.

102. Steinmetz, K.A.; Potter, J.D. Vegetables, fruit, and cancer prevention: A review. J. Am. Diet. Assoc. 1996, 96, 1027–1039.

103. Riboli, E.; Norat, T. Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. Am. J. Clin. Nutr. 2003, 78, 559S–569S.

104. Block, G.; Norkus, E.; Hudes, M.; Mandel, S.; Helzlouer, K. Which plasma antioxidants are most related to fruit and vegetable consumption? Am. J. Epidemiol. 2001, 154, 1113–1118.

105. Mente, A.; de Koning, L.; Shannon, H.S.; Anand, S.S. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. Arch. Intern. Med. 2009, 169, 659–669.

106. Ye, Z.; Song, H. Antioxidant vitamins intake and the risk of coronary heart disease: Meta-analysis of cohort studies. Eur. J. Cardiovasc. Prev. Rehabil. 2008, 15, 26–34.

107. Knekt, P.; Ritz, J.; Pereira, M.A.; O’Reilly, E.J.; Augustsson, K.; Fraser, G.E.; Goldbourt, U.; Heitmann, B.L.; Hallmans, G.; Liu, S.; et al. Antioxidant vitamins and coronary heart disease risk: A pooled analysis of 9 cohorts. Am. J. Clin. Nutr. 2004, 80, 1508–1520.

108. Lawlor, D.A.; Davey Smith, G.; Kundu, D.; Bruckdorfer, K.R.; Ebrahim, S. Those confounded vitamins: What can we learn from the differences between observational versus randomised trial evidence? Lancet 2004, 363, 1724–1727.

109. Maserejian, N.N.; Giovannucci, E.L.; McVary, K.T.; McKinlay, J.B. Dietary, but not supplemental, intakes of carotenoids and vitamin C are associated with decreased odds of lower urinary tract symptoms in men. J. Nutr. 2011, 141, 267–273.

110. Osganian, S.K.; Stampfer, M.J.; Rimm, E.; Spiegelman, D.; Hu, F.B.; Manson, J.E.; Willett, W.C. Vitamin C and risk of coronary heart disease in women. J. Am. Coll. Cardiol. 2003, 42, 246–252.

111. Agarwal, M.; Mehta, P.K.; Dwyer, J.H.; Dwyer, K.M.; Shircore, A.M.; Nordstrom, C.K.; Sun, P.; Paul-Labrador, M.; Yang, Y.; Merz, C.N. Differing relations to early atherosclerosis between vitamin C from supplements vs. food in the Los Angeles atherosclerosis Study: A prospective cohort study. Open Cardiovasc. Med. J. 2012, 6, 113–121.
112. Henriquez-Sanchez, P.; Sanchez-Villegas, A.; Doreste-Alonso, J.; Ortiz-Andrellucchi, A.; Pfimer, K.; Serra-Majem, L. Dietary assessment methods for micronutrient intake: A systematic review on vitamins. Br. J. Nutr. 2009, 102, S10–S37.

113. Thompson, F.E.; Byers, T. Dietary assessment resource manual. J. Nutr. 1994, 124, 2245S–2317S.

114. Dehghan, M.; Akhtar-Danesh, N.; McMillan, C.R.; Thabane, L. Is plasma vitamin C an appropriate biomarker of vitamin C intake? A systematic review and meta-analysis. Nutr. J. 2007, 6, 41.

115. Lykkesfeldt, J.; Poulsen, H.E. Is vitamin C supplementation beneficial? Lessons learned from randomised controlled trials. Br. J. Nutr. 2010, 103, 1251–1259.

116. Carr, A.C.; Pullar, J.M.; Moran, S.; Vissers, M.C.M. Bioavailability of vitamin C from kiwifruit in non-smoking males: Determination of ‘healthy’ and ‘optimal’ intakes. J. Nutr. Sci. 2012, 1, e14.

117. Franz, W.L.; Heyl, H.L.; Sands, G.W. Blood ascorbic acid level in bioflavonoid and ascorbic acid therapy of common cold. Br. J. Nutr. 1956, 162, 1224–1226.

118. Arminio, J.J.; Johnston, J.H.; Tebrock, H.E. Usefulness of bioflavonoids and ascorbic acid in treatment of common cold. J. Am. Med. Assoc. 1956, 162, 1227–1233.

119. Baird, I.M.; Hughes, R.E.; Wilson, H.K.; Davies, J.E.; Howard, A.N. The effects of ascorbic acid and flavonoids on the occurrence of symptoms normally associated with the common cold. Am. J. Clin. Nutr. 1979, 32, 1686–1690.

120. Stevenson, D.E.; Hurst, R.D. Polyphenolic phytochemicals—Just antioxidants or much more? Cell. Mol. Life Sci. 2007, 64, 2900–2916.

121. Gao, Y.; Li, W.; Jia, L.; Li, B.; Chen, Y.C.; Tu, Y. Enhancement of (−)-epigallocatechin-3-gallate and theaflavin-3,3′-digallate induced apoptosis by ascorbic acid in human lung adenocarcinoma SPC-A-1 cells and esophageal carcinoma Eca-109 cells via MAPK pathways. Biochem. Biophys. Res. Commun. 2013, 438, 370–374.

122. Calero, C.I.; Beltran Gonzalez, A.N.; Gasulla, J.; Alvarez, S.; Evelson, P.; Calvo, D.J. Quercetin antagonism of GAB receptors is prevented by ascorbic acid through a redox-independent mechanism. Eur. J. Pharmacol. 2013, doi:10.1016/j.ejphar.2013.07.044.

© 2013 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).