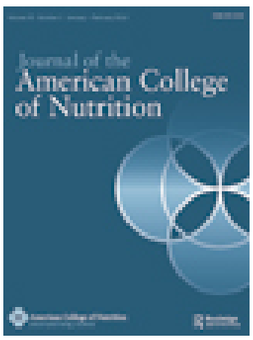

Journal of the American College of Nutrition

ISSN: 0731-5724 (Print) 1541-1087 (Online) Journal homepage: https://www.tandfonline.com/loi/uacn20

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To cite this article: Malkanthi Evans, Najla Guthrie, H. Kelly Zhang, William Hooper, Andrew Wong & Annahita Ghassemi (2019): Vitamin C Bioequivalence from Gummy and Caplet Sources in Healthy Adults: A Randomized-Controlled Trial, Journal of the American College of Nutrition, DOI: 10.1080/07315724.2019.1684398

To link to this article: https://doi.org/10.1080/07315724.2019.1684398

Published online: 20 Nov 2019.

JOURNAL OF THE AMERICAN COLLEGE OF NUTRITION
https://doi.org/10.1080/07315724.2019.1684398

Vitamin C Bioequivalence from Gummy and Caplet Sources in Healthy Adults: A Randomized-Controlled Trial

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ABSTRACT

Background: The efficacy of Vitamin C (L-ascorbic acid) supplementation can be assessed by uptake into the blood and retention in leukocytes. Vitafusion® Power C gummy is an alternative vitamin C source which may exhibit similar bioavailability to comparator caplets.

Objective: The objective of this study was to evaluate the bioequivalence of vitamin C from a vitafusion® Power C gummy formulation and a comparator caplet in healthy adults.

Methods: Thirty healthy men and women, 34.0 ± 11.4 years of age and Body Mass Index (BMI) 24.5 ± 3.6 kg/m^2 completed the randomized examiner-blind, comparator controlled, cross-over trial with two sequences: gummy (1000 mg) to caplet (1000 mg) or caplet to gummy. Intake of foods fortified with Vitamin C was restricted 7 days prior to each dosing. Blood samples were collected pre-dose and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h post-dose for plasma and leukocytes; and urine was collected pre-dose and between 0-2, 2-4, 4-8, 8-12 and 12-24 h post-dose for L-ascorbic acid analysis.

Results: Vitafusion® Power C gummy and comparator caplet demonstrated similar plasma absorption profiles as there were no significant differences in plasma L-ascorbic acid total Area Under the Curve $(AUC)_{0\text{-}24h}$, and T_{max} between gummy and caplet. The caplet did elicit a significantly higher C_{max} than the gummy ($p < 0.05$), however, the difference was numerically small. Leukocyte L-ascorbic acid total $AUC_{0\text{-}24h}$ and C_{max} were not significantly different between gummy and caplet, however T_{max} of the gummy group was significantly longer ($p = 0.012$). Urinary L-ascorbic acid levels were also not significantly different between gummy and caplet. There were no serious adverse events and safety parameters remained within normal clinical range for both products.

Conclusion: Vitafusion® Power C gummy exhibited similar Vitamin C absorption and bioavailability to a comparator caplet in healthy adults and were considered bioequivalent.

Abbreviations: AE: adverse event; ANOVA: repeated measures analysis of variance; AUC: area under the curve; BMI: body mass index; Ca: calcium; C_{max}: maximum concentration; CPT: cell preparation tube; HPLC: high performance liquid chromatography; iAUC: Incremental Area Under Curve; Mg: magnesium; NHANES: National Health and Nutrition Examination Survey; NHPD: Natural Health Product Directorate; PBS: phosphate buffered saline; PK: pharmacokinetic; QI: qualified investigator; RCF: relative centrifugal force; RDI: recommended dietary intake; T_{max}: time to maximum concentration

ARTICLE HISTORY
Received 1 May 2019
Accepted 19 October 2019

KEYWORDS
Vitamin C; L-ascorbic acid; vitafusion® Power C gummy; absorption; bioavailability; bioequivalence; multivitamin

Introduction

Vitamin C is an essential, water-soluble micronutrient that plays a critical role in immunity (1, 2). Vitamin C functions as a co-factor for numerous enzymes, provides antioxidant protection to plasma lipids and is required for the synthesis of amino acid derived macromolecules, collagen, neurotransmitters, and neuropeptide hormones (3, 4). Immune cells possess transporters that actively internalize vitamin C during times of infection (5). Recent studies also suggest that vitamin C may confer protection against disease conditions (6). To function as an effective antioxidant, high levels of vitamin C must be obtained and retained in the body (3, 7).

Vitamin C is readily obtained from numerous food sources such as citrus fruits, blackcurrant, guava, kiwi fruit, broccoli, and sprouts, however, it is a labile micronutrient. As a result, its concentration in food sources can be affected by season, transport, storage time and cooking practices. Studies demonstrating the health benefits of vitamin C have encouraged people to supplement their diets (5, 8). The recommended dietary allowance (RDA) for vitamin C ranges from 75 to 120 mg/day in healthy adults and is most commonly obtained from the diet. Consumption of vitamin C supplements is an efficient way of increasing vitamin C levels in the body when dietary sources are unable to meet the recommended requirements. National Health and Nutrition

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Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/uacn.
Source of Support: Church & Dwight Co., Inc., Ewing NJ, USA, 08628
Trial registration number: NCT# 03562988
URL of registration: https://clinicaltrials.gov/

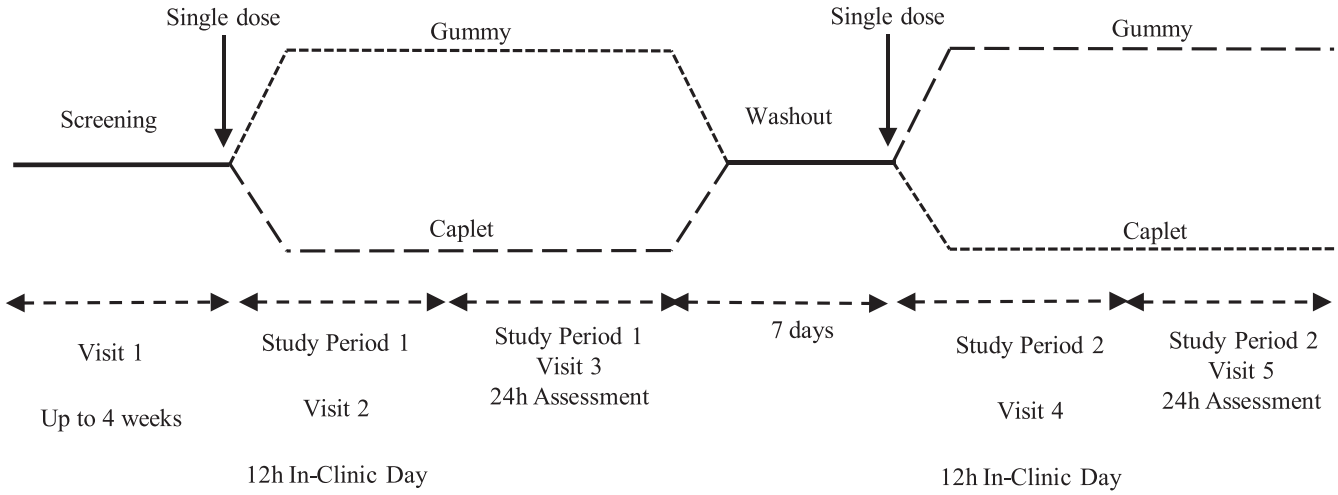

Figure 1. Study design. Thirty-five participants were enrolled in a randomized, examiner-blind, comparator-controlled, cross-over study consisting of two 24-hour study periods separated by a 7-day washout. Each study period consisted of blood and urine sampling conducted at regular time points.

Examination Survey (NHANES) data from 1999 to 2001 suggest that approximately 35% adults in the U. S. consume multivitamins containing vitamin C and about 12% of adults consume additional vitamin C supplements. On average vitamin C consumption in adult males is 105.2 mg/day and 83.6 mg/day in adult females. A recommendation of 1000 mg/day of vitamin C has not only been shown to be safe (9), but higher oral intake of vitamin C increases bioavailability, which can lead to optimal health and avoidance of several disease conditions (9). Additionally, a recent double-blind, placebo-controlled bioavailability study by Mitmesser et al. showed a dose of 1000 mg/day resulted in a favorable percent change in leukocyte of vitamin C concentration (10).

Plasma L-ascorbic acid concentrations reflect the amount of vitamin C acutely absorbed from the digestive tract (11). However, the efficacy of absorption is determined by its uptake into cells, such as leukocytes, and tissues (11, 12). Both plasma and leukocyte L-ascorbic acid concentrations have been found to correlate with dietary intakes, however, leukocyte L-ascorbic acid is considered to more accurately reflect long-term intake (11). Urinary L-ascorbic acid depicts the elimination of vitamin C from circulation and is measured in conjunction with plasma and leukocyte to collectively assess storage, excretion, and ultimately the pharmacokinetic profile of vitamin C. While tissues stores have been shown to reach saturation at approximately 60 mg/day (8), and high urinary vitamin C excretion is expected at high doses, total body content of vitamin C can range from 300 to 2000 mg (13). Highest concentrations are maintained in leukocytes and tissues including eyes, brain, and adrenal glands supporting the essentiality of adequate vitamin C intake.

It has been suggested that different delivery forms, including tablets, liquids, powders, or gummies, may potentially alter the bioavailability of the supplement (14). Most recently, in a cross-over study in healthy adults ($n = 31$) comparing vitamin D gummies and tablets, it was found that vitamin D gummies had greater bioavailability compared to tablets with higher vitamin D serum concentrations over a 48-hour period (15). Vitamin C gummy formations provide a convenient way for individuals to meet the RDA of vitamin C, however information about the bioequivalence of vitamin C from gummy formulations are lacking. Therefore, we performed a randomized, examiner-blind, cross-over study to assess the bioequivalence of vitamin C from vitafusion® Power C gummy formulation and a comparator caplet in healthy adults after a single dose. We hypothesize that the bioavailability of the gummy formulation and comparator caplet formulation of vitamin C supplements will be similar.

Materials and methods

Study design

This single-center, randomized, examiner-blind, comparator-controlled, cross-over bioequivalence study was conducted at KGK Science Inc., London, ON, Canada. The study consisted of two, 24-hour study periods each preceded by a 7-day washout period (Figure 1).

During the screening visit, the volunteers' medical histories and concomitant therapies were reviewed, and their eligibility assessed; height, weight, blood pressure, and heart rate were measured, and BMI calculated. Blood was sampled for hematology, clinical chemistry, and liver and kidney function tests. All volunteers were counseled to avoid the consumption of vitamin C supplements, multivitamins containing vitamin C, citrus foods and juices, and foods and beverages fortified with vitamin C for at least 7 days prior to baseline and during the study. All volunteers were provided with a list of low vitamin C foods that were permitted for consumption during the washout period. Foods containing less than 2.4 mg or 4% of the RDA of vitamin C were allowed during the washout period.

Eligible participants returned to the clinic for their study period 1 (baseline) and were randomized into one of two treatment sequences: gummy to caplet or caplet to gummy by a blinded investigator per the order of the randomization list generated by www.randomization.com. The investigational product was randomized and coded by an un-blinded person not involved in data collection or analysis. This study was

single blinded (examiner only) due to the obvious difference in product form (gummy vs. caplet). Investigators, other site personnel, and participants were blinded to the product.

On the first day of each study period, 7-day food records were reviewed to ensure compliance to food requirements, and pre-dose blood and urine samples were collected. Participants were required to consume the investigational product or comparator as per their allocated treatment sequence under the supervision of a clinical coordinator to ensure compliance. Post-dose, blood samples were collected at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h for the analysis of plasma and leukocyte L-ascorbic acid concentration. Urine was collected between 0–2, 2–4, 4–8, 8–12, and 12–24 h for the analysis of L-ascorbic acid concentrations. Repeated blood or urine samples were collected to examine the pharmacokinetics in blood and urine of the investigational product or comparator. Line graphs were constructed showing individual and mean concentrations of plasma, leukocyte, and urine L-ascorbic acid. This was used to calculate an area under the curve over the 24 hour collection period (AUC_{0-24h}), incremental AUC over 24 hours ($iAUC_{0-24h}$), maximum L-ascorbic acid concentration (C_{max}), time to maximum concentration (T_{max}), terminal disposition rate constant, and terminal half life of L-ascorbic acid. All participants were required to remain in the clinic from pre-dose until the 12-h post-dose sampling. No food was provided until after 2 h. Participants were provided with standardized meals devoid of vitamin C: breakfast after 2 h, lunch after 5 h and supper between 6 and 12-h post-dose blood sampling. Participants were provided with containers for the 12–24 h urine collection. Participants were counseled to adhere to a low vitamin-C diet prior to the 24-h clinic visit. Participants returned to the clinic the next day for the 24-h sampling. Participants were counseled to comply with the low vitamin C food requirement during the washout period and to record their dietary intake to establish dietary compliance.

Participants

Healthy male and female adults from Southwestern Ontario between 18 and 55 years of age were recruited into the study. Inclusion criteria comprised of healthy volunteers with a BMI 18.5–29.5 kg/m^2 who were nonsmokers and deemed healthy as per physical exam, hematology, clinical chemistry, liver and kidney function. Volunteers were excluded if: had a history of or had gastrointestinal disease, history of malabsorption, high blood pressure, cancers, metabolic disease, history of kidney stones, bleeding disorders, or anemia, were immunocompromised, were on anticoagulants, non-steroidal anti-inflammatory drugs, beta-blockers, tetracycline antibiotics, immunosuppressants, barbiturates, antidepressants, diuretics or nitrate medications, marijuana, aluminum, iron, and proton pump inhibitors. The first potential participant was screened on August 4, 2017; the first participant was randomized into the study on September 7, 2017 and the last participant's final visit was completed on November 30, 2017.

Investigational product

The investigational product-vitafusion® Power C gummy (containing: 149.21 mg Vitamin C per gummy) and the comparator-*Nature Made*® vitamin C caplet (containing: 513.95 mg vitamin C per caplet) were stored at room temperature and protected from light until administration. The investigational product and comparator were chosen as they contained the same form of vitamin C, ascorbic acid, and both contained additional rose hips. Participants were administered 6.75 gummies (total dose of 1007.2 mg ascorbic acid) or 2 caplets (total dose of 1027.9 mg ascorbic acid) as per their allocated treatment sequence. All participants were required to consume 250 mL of water with both products. Product analysis was conducted to measure the vitamin C content prior to study start to determine dosage of gummy and caplet; and at the end of the study to ensure product stability through the study.

Sample preparation for plasma and leukocyte L-ascorbic acid analyses

Blood was collected in 8 mL sodium-heparin cell preparation tubes (CPT)^{TM} (BD Vacutainer, Missisauga, ON) for plasma and leukocyte L-ascorbic acid analysis. The tubes were centrifuged at 1500 relative centrifugal field (RCF) for 20 min at room temperature, and plasma was separated and stored at −80 °C until further analysis. The mononuclear cell layer was isolated and washed twice with phosphate buffered saline (PBS) (without calcium $(Ca)^{++}$ or magnesium $(Mg)^{++}$) by centrifuging at 300 RCF for 15 min at 4 °C. The supernatant was discarded, and the cell pellet was resuspended in PBS (without Ca^{++} or Mg^{++}) and frozen at −80 °C until further analysis.

Sample preparation for urinary L-ascorbic acid analysis

Urine collected from pre-dose and each post-dose time interval was stored at 4 °C until the end of each collection period. At the end of each collection period, total urine volumes collected at each post-dose time interval were recorded, and the urine container agitated to ensure homogeneity, a sample was retrieved and centrifuged at 1000 RCF for 20 min at 4 °C. An aliquot of the supernatant was transferred to a cryovial and stored at −80 °C until further analysis.

Plasma L-ascorbic acid analysis

Plasma samples were analyzed at LifeLabs (Toronto, ON). Sulfosalicylic acid was added to the samples to precipitate proteins and to extract ascorbic acid. The L-ascorbic acid in the supernatant was then quantified by high performance liquid chromatography (HPLC). Briefly, 50 µL of the supernatant was injected into a reverse phase Zorbax Eclipse Plus C18 column (Agilent, Santa Clara, CA, USA), separated using metaphosphoric acid as the mobile phase and detected using an electrochemical detector.

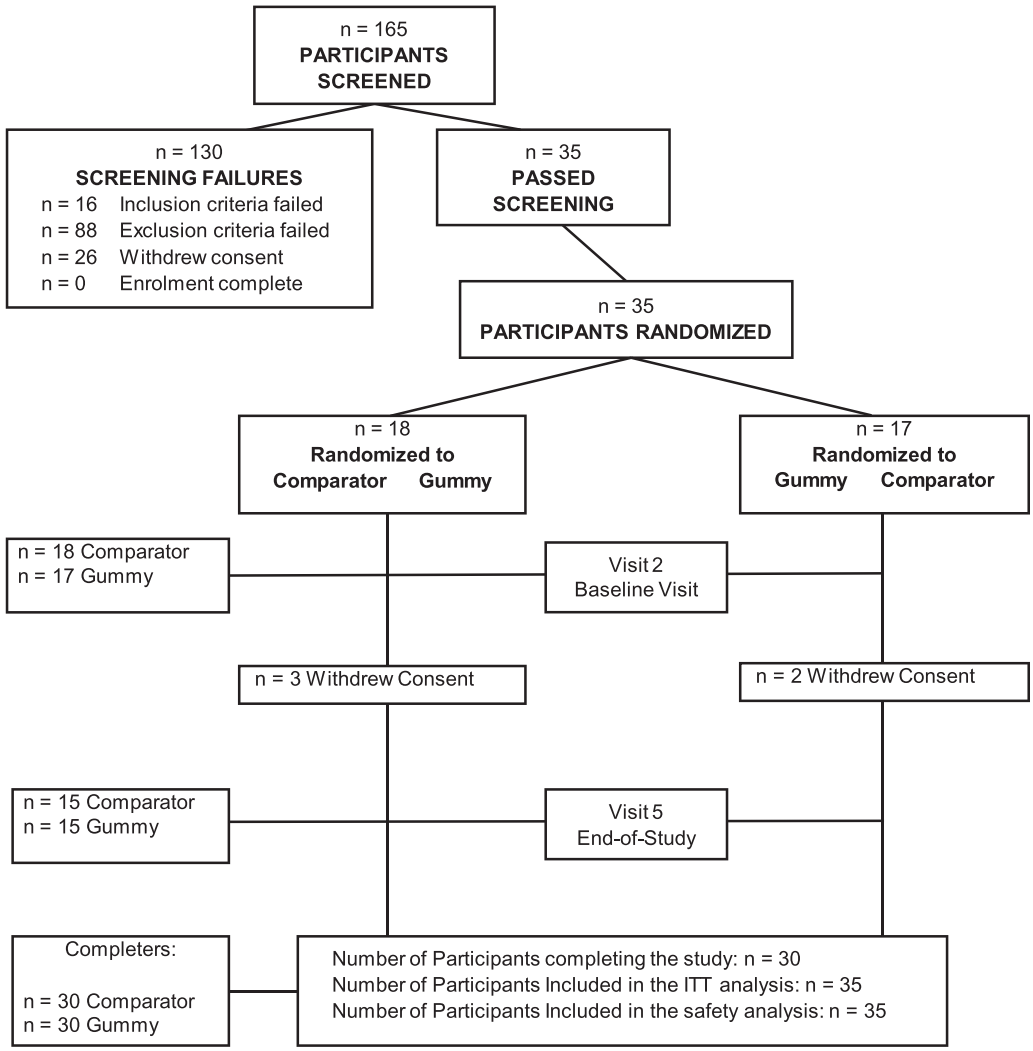

Figure 2. Participant disposition. Of 165 individuals screened, 35 participants were eligible and randomized at baseline into either caplet → gummy or gummy → caplet sequences. Five participants who did not complete the study had withdrawn consent. The intention-to-treat and safety populations are defined as all 35 participants who received either study products and on whom any post-randomization information is available; the per-protocol population comprises of the 30 participants who completed all visits and procedures.

Leukocyte and urinary L-ascorbic acid analysis

Mononuclear leukocyte cell samples and urine samples were analyzed at Eve Technologies (Calgary, AB, Canada). Briefly, leukocyte and urine samples were filtered and the L-ascorbic acid in the filtrate was measured using a colorimetric assay kit from Abcam (Catalog number: ab65346) (Cambridge, MA, USA). L-Ascorbic acid standards and test samples were prepared in 96-well plates as outlined in the kit protocol. Diluted catalyst and the reaction mix containing ascorbic acid assay buffer, probe, and enzyme mix were then added to all the wells, and L-ascorbic acid was quantified in a microplate reader at 570 nm.

Safety

Safety outcomes included vital signs (resting heart rate and blood pressure), BMI, complete blood counts, electrolytes, creatinine, aspartate aminotransferase, alanine aminotransferase, bilirubin, as well as the incidence of any adverse events. Blood samples were analyzed at Life Labs (London, ON, Canada). Adverse events reported by the participants were categorized using the Medical Dictionary for Regulatory Activities version 17.0.

Statistics

A total of 30 participants completed the study. Since this was a bioequivalence study, no sample size calculation was conducted, and no changes were made to pre-declared primary or secondary endpoints of the study. The pharmacokinetics (PK) parameters included total AUC_{0-24h}, $iAUC_{0-24h}$, C_{max} (maximum concentration), $T_{max,}$ (time to maximum concentration), terminal disposition rate constant, and terminal half life. Possible differences between groups for the PK parameters, the concentrations collected at each time point, and for the changes from 0 h (pre-ingestion) were assessed by repeated measures analysis of variance (ANOVA). The model included fixed effects for group, sequence and period with participant as a random effect. Assumptions of normality of residuals were investigated for each statistical model. If the normality assumption was

Table 1. Demographics for participants in the per-protocol (PP) population ($n = 30$).

	Gummy → Caplet ($n = 15$)	Caplet → Gummy ($n = 15$)	p-value$^{\Delta}$
Age			0.380
Mean ± SD	32.1 ± 10.5	35.9 ± 12.3	
Median (Min–Max)	28 (21–51)	37 (19–54)	
BMI (kg/m^2) at Screening			0.333
Mean ± SD	23.9 ± 4.0	25.2 ± 3.1	
Median (Min–Max)	24 (17.9–30.7)	25.3 (19.6–30.6)	
Body weight (kg) at Screening			0.978
Mean ± SD	68.8 ± 13.4	68.7 ± 7.1	
Median (Min–Max)	70.4 (48.1–92.5)	68 (59.7–83.4)	
Gender [n (%)]			0.427
Female	9 (60%)	12 (80%)	
Male	6 (40%)	3 (20%)	
Alcohol consumption status [n (%)]			0.260
None	3 (20%)	1 (7%)	
Occasional	5 (33%)	10 (67%)	
Weekly	7 (47%)	4 (27%)	
Smoking status [n (%)]			1.000
Ex-smoker > 1 year	1 (7%)	1 (7%)	
No	14 (93%)	14 (93%)	
Race [n (%)]			0.522
East Asian	0 (0%)	1 (7%)	
Eastern European White	2 (13%)	1 (7%)	
Middle Eastern	2 (13%)	0 (0%)	
South American	2 (13%)	1 (7%)	
Western European White	9 (60%)	12 (80%)	

Legend: n = number; SD = standard deviation; Min = minimum; Max = maximum, BMI = body mass index.
$^{\Delta}P$ values by Fisher's exact test.

rejected at the 1% level by the Shapiro–Wilk test then the data were analyzed using a rank transformation (16). Within group changes from 0 h were assessed by the paired t-test or Wilcoxon signed rank test, depending on the distribution of the data.

Ethics

The study was approved by the Natural Health Product Directorate (NHPD), Health Canada, Ottawa, ON and Institutional Review Board, Aurora, ON and conducted in accordance with the Guideline for Good Clinical Practice (International Conference on Harmonization) and ethical principles according to the Declaration of Helsinki. The study was adequately explained and written informed consent was obtained from each participant prior to initiation of any study related procedures. The trial was registered with ClinicalTrials.gov (Identifier: NCT# 03562988).

Results

Participants

A total of 165 participants were screened, and 35 eligible participants were enrolled in the study with 18 participants in the caplet to gummy sequence; and 17 participants in the gummy to caplet sequence. There were no significant differences in the demographics and baseline characteristics between the two treatment sequences in age, weight, BMI, gender, race, alcohol consumption, and smoking status. No premature unblinding was required during the study. Thirty participants completed the study (Figure 2 and Table 1).

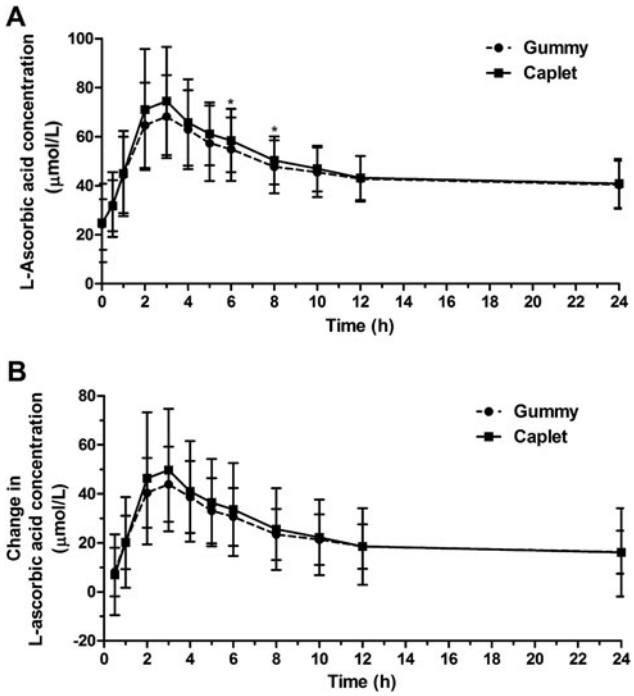

Figure 3. Plasma L-ascorbic acid (A) absolute concentrations (mean ± SD, µmol/L) and (B) change from baseline concentrations at 0 h, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h post dose (mean ± SEM, µmol/L) for participants in the per protocol population ($n = 30$). Plasma L-ascorbic acid concentration (A) was significantly higher for the caplet group than the gummy group at 6 h ($p = 0.021$) and 8 h ($p = 0.042$). Changes from baseline concentration (B) did not differ between groups at any time point. * Denotes statistically significant difference ($p < 0.05$) between groups.

Plasma L-ascorbic acid analysis

The expected plasma response to acute consumption of vitamin C formulations was seen with both products (Figure 3A). Statistically significant increase from baseline

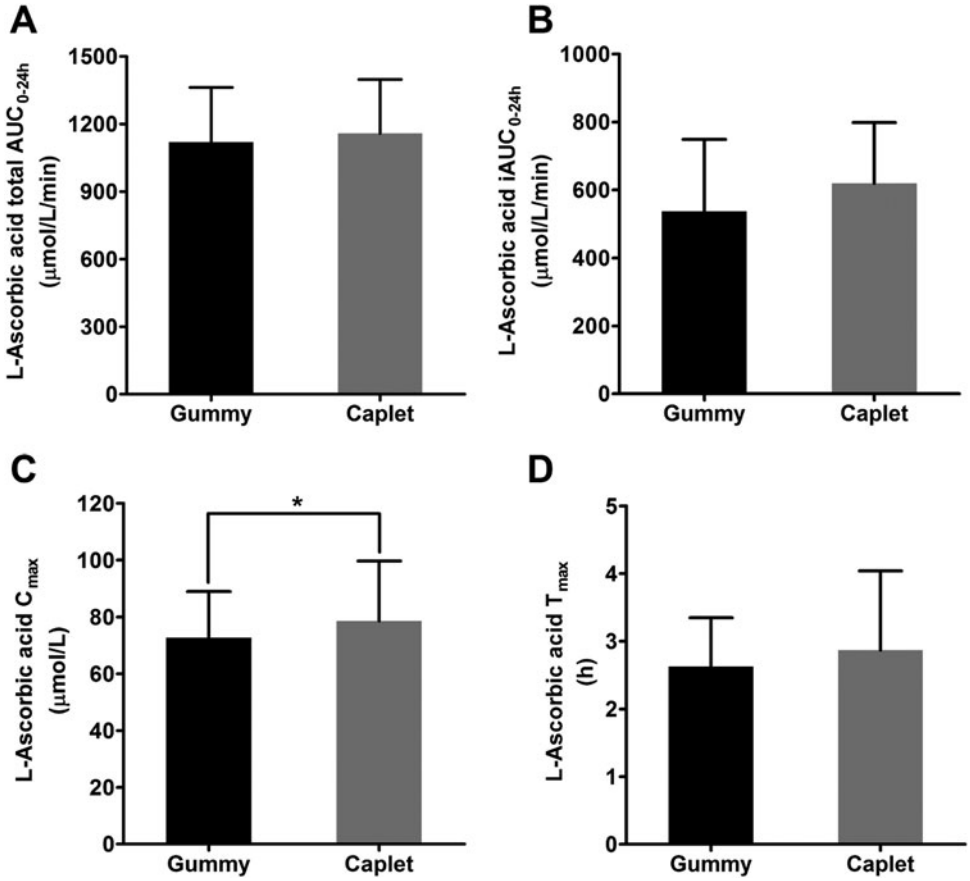

Figure 4. Plasma L-ascorbic acid (A) Total area under the curve $(AUC)_{0-24h}$ (B) $iAUC_{0-24h}$, (C) maximum concentration (C_{max}), and (D) time to maximum concentration (T_{max}) following ingestion of gummy or comparator for participants in the per protocol population ($n = 30$). Values are means ± SDs. There was no statistical significance between groups in L-ascorbic acid total AUC_{0-24h} (A), $iAUC_{0-24h}$ (B), and T_{max} (D). C_{max} (C) for the caplet group was significantly higher than that of the gummy group ($p = 0.031$). * Denotes statistically significant difference ($p < 0.05$) between groups. AUC = area under the curve; C_{max} = maximum concentration; T_{max} = time to maximum concentration.

Table 2. Plasma L-ascorbic acid concentrations pre-ingestion (0 h) and pharmacokinetic parameters.

	Gummy Mean ± SD (*n*) Median (Min–Max)	Comparator Mean ± SD (*n*) Median (Min–Max)	Between-group *p*-value*
0 h Pre-ingestion (µmol/L)	24.2 ± 10.3 (30) 23.5 (12–57)	24.8 ± 16.0 (30) 23 (5–97)	0.865
$iAUC_{0-24h}$ (µmol·min/L)	537.70 ± 211.73 (30) 526.13 (121.25–1034.00)	619.62 ± 178.88 (30) 592.63 (273.45–1120.25)	0.0580
Total AUC_{0-24h} (µmol·min/L)	1119.13 ± 244.10 (30) 1115.50 (685.00–1730.00)	1157.88 ± 240.68 (30) 1165.38 (578.50–1627.75)	0.1313
C_{max} (µmol/L)	72.70 ± 16.26 (30) 68.50 (40.00–114.00)	78.63 ± 21.13 (30) 76.00 (46.00–127.00)	0.0313
T_{max} (h)	2.63 ± 0.72 (30) 3.00 (2.00–5.00)	2.87 ± 1.17 (30) 3.00 (1.00–6.00)	0.6020 (r)
Terminal disposition rate constant	0.007 ± 0.005 (30) 0.006 (0.000–0.020)	0.009 ± 0.008 (30) 0.006 (0.000–0.030)	0.3292 (r)
Terminal half-life	470.60 ± 882.80 (30) 107.88 (35.01–3652.65)	383.75 ± 1040.33 (30) 118.64 (22.89–5628.18)	0.3292 (r)

Legend: *n* = number; SD = standard deviation; Min = minimum; Max = maximum; AUC = area under the curve.
*Between group *p*-values were generated by repeated measures ANOVA with group, sequence and period as fixed effects and subject as a random effect. (r) indicates ranked transformation was used.

plasma L-ascorbic acid levels occurred at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h ($p < 0.001$) and C_{max} was seen at 3 h post consumption for both products (Figure 3A). In addition, plasma L-ascorbic acid concentration was significantly higher for the caplet group than the gummy group at 6 h ($p = 0.021$) and 8 h ($p = 0.042$) post consumption (Figure 3A). The change in plasma L-ascorbic acid concentration from baseline was also not significantly different between the gummy and caplet group at any time point (Figure 3B).

There were no significant differences in L-ascorbic acid total AUC_{0-24h} (Figure 4A) $iAUC_{0-24h}$ (Figure 4B), and T_{max} (Figure 4D) between the gummy and caplet groups. The L-ascorbic acid C_{max} of the gummy was significantly lower ($p = 0.031$) than the caplet (Figure 4C and Table 2). There were no significant differences in the L-ascorbic acid terminal disposition rate constant, and terminal half-life between the gummy and the caplet groups (Table 2).

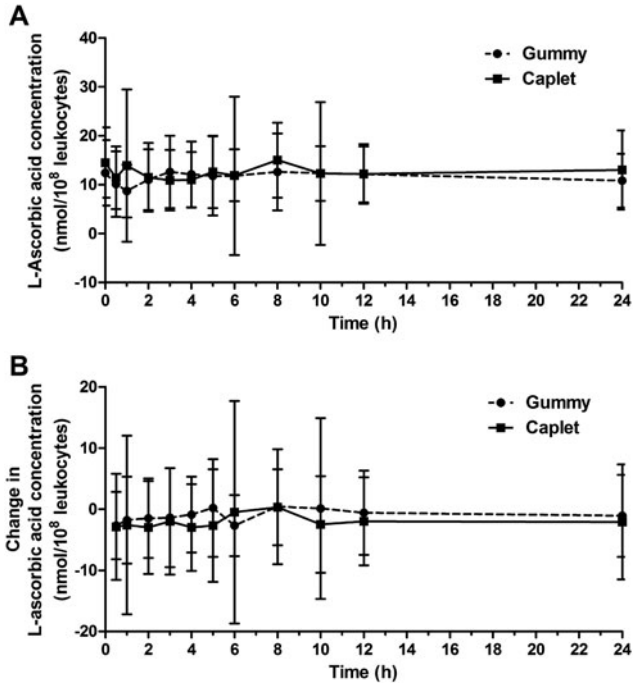

Figure 5. Leukocyte L-ascorbic acid (A) concentrations (mean ± SD, µmol/L) and (B) change from baseline concentrations at 0 h, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h post dose (mean ± SEM, µmol/L) for participants in the per protocol population ($n = 30$). Leukocyte L-ascorbic acid concentration (A) and change from baseline concentration (B) were not significantly different between the two groups at any time point.

Leukocyte L-ascorbic acid analysis

There were no significant differences in leukocyte L-ascorbic acid concentrations between the gummy and caplet groups (Figure 5A). The change in leukocyte L-ascorbic acid concentration from baseline were also not significantly different between the gummy and caplet group at any time point (Figure 5B).

There were no significant differences in L-ascorbic acid total AUC_{0-24h} (Figure 6A), $iAUC_{0-24h}$ (Figure 6B), C_{max} (Figure 6C) between the gummy and caplet groups. The L-ascorbic acid T_{max} of the gummy group was significantly longer ($p = 0.012$) than the caplet (Figure 6D and Table 3). There were no significant differences in the L-ascorbic acid terminal disposition rate constant, and terminal half-life between the gummy and the caplet groups (Table 3).

Urine L-ascorbic acid analysis

No significant between-group differences were found in absolute concentration (Figure 7A) and change from baseline concentration (Figure 7B) in urinary L-ascorbic acid at any of the time intervals. Statistically significant within group changes in L-ascorbic acid levels from baseline occurred at all time-intervals ($p < 0.001$) and peak elimination was seen between 2–4 h post consumption in gummy

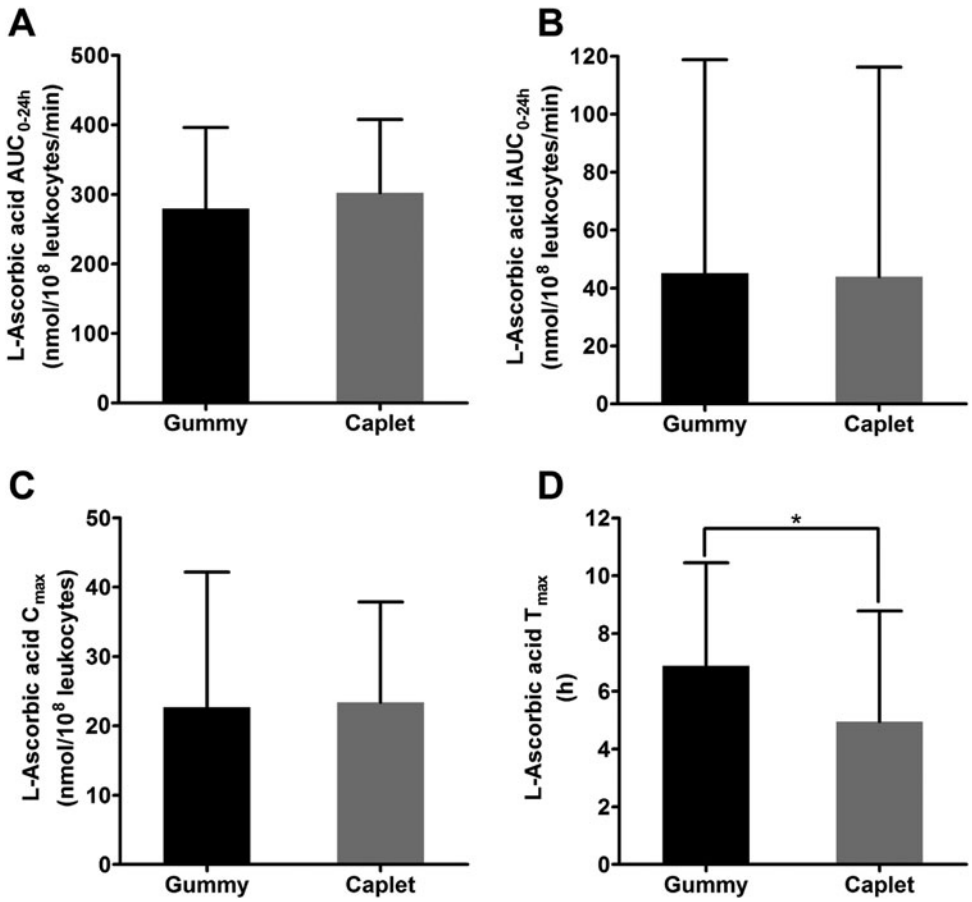

Figure 6. Leukocyte L-ascorbic acid (A) total area under the curve $(AUC)_{0-24h}$ (B) $iAUC_{0-24h}$, (C) maximum concentration (C_{max}), and (D) time to maximum concentration (T_{max}) for participants in the per protocol population ($n = 30$). Values are means ± SDs. There was no statistical significance between groups in L-ascorbic acid total AUC_{0-24h} (A), $iAUC_{0-24h}$ (B), and C_{max} (C). T_{max} (D) of the gummy group was significantly higher than that of the comparator group ($p = 0.012$). * Denotes statistically significant difference ($p < 0.05$) between groups. AUC = area under the curve; C_{max} = maximum concentration; T_{max} = time to maximum concentration.

Table 3. Leukocyte L-ascorbic acid concentrations pre-ingestion (0 h) and pharmacokinetic parameters.

	Gummy Mean ± SD (*n*) Median (Min–Max)	Comparator Mean ± SD (*n*) Median (Min–Max)	Between-group *p*-value*
0 h Pre-ingestion (μmol/L)	13.16 ± 7.17 (30) 13.12 (1.57–30.15)	13.79 ± 6.92 (30) 13.26 (0.00–31.57)	0.6357
iAUC$_{0-24h}$ (nmol/10^8 leukocytes)	45.15 ± 73.70 (30) 12.52 (0.00–308.19)	43.90 ± 72.36 (30) 14.66 (0.00–352.25)	0.7806 (r)
Total AUC$_{0-24h}$ (nmol·min/10^8 leukocytes)	279.41 ± 116.70 (30) 269.69 (41.01–579.86)	302.05 ± 105.81 (30) 307.67 (100.07–531.34)	0.4175
C$_{max}$ (nmol/10^8 leukocytes)	22.71 ± 19.46 (30) 16.45 (7.22–93.21)	23.38 ± 14.49 (30) 19.36 (7.96–80.56)	0.3716 (r)
T$_{max}$ (h)	6.88 ± 3.57 (30) 7.00 (0.50–12.00)	4.95 ± 3.83 (30) 4.50 (0.50–12.00)	0.0123 (r)
Terminal disposition rate constant	0.021 ± 0.027 (30) 0.011 (0.000–0.116)	0.021 ± 0.024 (30) 0.011 (0.000–0.109)	0.8649 (r)
Terminal half-life	326.73 ± 1280.13 (30) 64.06 (5.97–7078.11)	665.99 ± 2368.41 (30) 62.04 (6.36–12128.34)	0.8649 (r)

Legend: *n* = number; SD = standard deviation; Min = minimum; Max = maximum; AUC = area under the curve
*Between group *p*-values were generated by repeated measures ANOVA with group, sequence and period as fixed effects and subject as a random effect. (r) indicates ranked transformation was used.

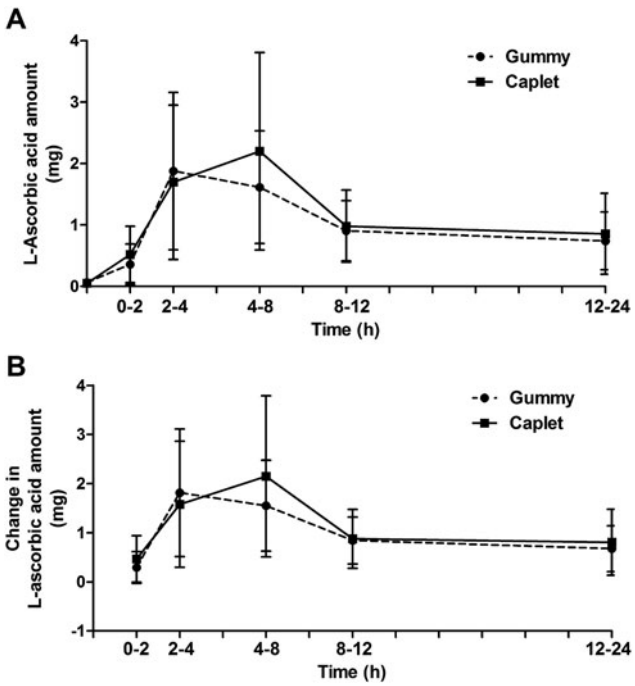

Figure 7. Urinary L-ascorbic acid (A) amount (mean ± SD) and (B) change from baseline amount (mean ± SEM) at pre-dose (0 h) and at time intervals 0–2, 2–4, 4–8, 8–12, and 12–24 h post-dose for participants in the per protocol population (*n* = 30). Urinary L-ascorbic acid amount (A) and change from baseline amount (B) were not significantly different between the two groups at any time point.

group and 4–8 h post consumption in the caplet group (Figure 7B and Table 4).

Safety

A total of 11 adverse events (AEs) were reported by nine participants during the study. Of these, seven AEs were reported by six participants who consumed the gummy and four AEs were reported by three participants who consumed the caplet. Of the seven AEs reported by the gummy group, two (absolute neutrophil count abnormal and sleepiness) were categorized as possibly related to the product and the rest were categorized as not related or unlikely related to the product. Of the four AEs that were reported by the caplet group, one was categorized as possibly related to the product (nausea) and the rest of the AEs were categorized as not related or unlikely related to the product. All AEs were resolved by end of study. There were no serious adverse events reported during the study.

There were no clinically relevant differences between gummy and caplet groups in hematology, clinical chemistry, vital signs and anthropometric measurements. Although there were statistically significant differences in pre-dose to post-dose changes in participants' potassium levels and systolic blood pressure ($p = 0.047$; $p = 0.046$, respectively) between the gummy and caplet groups, these values remained within normal clinical ranges for both groups and were deemed to be clinically non-significant by the qualified investigator (QI).

Discussion

This study demonstrated that vitamin C consumed in the form of a vitafusion® Power C gummy, produced similar L-ascorbic acid concentrations in plasma and leukocytes relative to a comparator caplet in healthy adults. In addition, vitamin C from vitafusion® Power C gummy was excreted in the urine as efficiently as vitamin C from a caplet. In this study, all participants received a single 1000 mg dose of vitamin C in gummy or caplet form. Pre-dose L-ascorbic acid levels in plasma showed limited variability in the completers' population, indicating all participants had similar baseline levels of L-ascorbic acid prior to the start of both sequences.

Plasma absorption profiles for the gummy and caplet were not significantly different, suggesting similar bioavailability. Peak concentrations were achieved at 3-h post consumption of both products. Total AUC$_{0-24h}$ and iAUC$_{0-24h}$ determine the extent of absorption and bioequivalence of L-ascorbic acid over the 24-hour period following a single dose. Total AUC$_{0-24h}$ describes the total bioavailability of a product following a single dose, while iAUC$_{0-24h}$ describes the "incremental" bioavailability. Plasma L-ascorbic acid total AUC$_{0-24h}$ and iAUC$_{0-24h}$ elicited by the gummy was

Table 4. Mean urinary L-ascorbic acid concentrations at baseline (pre-ingestion, 0 h) and change from baseline at time intervals 0–2, 2–4, 4–8, 8–12, and 12–24 h.

	Gummy **Mean ± SD (*n*) Median (Min–Max)** **Within-group *p*-value**	Comparator **Mean ± SD (*n*) Median (Min–Max)** **Within-group *p*-value**	**Between-group *p*-value***
0 h Pre-ingestion (μmol/L)	0.0648 ± 0.0510 (30) 0.0419 (0.0098–0.2071)	0.0529 ± 0.0318 (28) 0.0457 (0.0002–0.1547)	0.3004
Change from baseline to 0–2 h	0.29 ± 0.32 (30) 0.25 (−0.01–1.30) **$p < 0.001^{\dagger}$**	0.47 ± 0.47 (28) 0.28 (−0.04–1.82) **$p < 0.001^{\dagger}$**	0.0983
Change from baseline to 2–4 h	1.81 ± 1.30 (30) 1.42 (0.35–5.39) **$p < 0.001^{\dagger}$**	1.58 ± 1.28 (26) 1.2751 (−0.02–5.97) **$p < 0.001^{\dagger}$**	0.4393
Change from baseline to 4–8 h	1.55 ± 0.92 (30) 1.45 (0.24–3.65) **$p < 0.001^{\delta}$**	2.15 ± 1.64 (29) 1.94 (0.15–7.29) **$p < 0.001^{\dagger}$**	0.0959
Change from baseline to 8–12 h	0.84 ± 0.48 (30) 0.80 (−0.01–1.81) **$p < 0.001^{\delta}$**	0.88 ± 0.60 (29) 0.80 (−0.13–2.71) **$p < 0.001^{\delta}$**	0.8050
Change from baseline to 12–24 h	0.68 ± 0.47 (30) 0.62 (0.094–1.89) **$p < 0.001^{\delta}$**	0.81 ± 0.67 (30) 0.62 (0.02–2.90) **$p < 0.001^{\dagger}$**	0.1926

Legend: n = number; SD = standard deviation; Min = minimum; Max = maximum.
*Between group p-values were generated by repeated measures ANOVA with group, sequence and period as fixed effects and subject as a random effect for each time point.
†Within group comparisons were made using Wilcoxon signed rank test.
δWithin group comparisons were made using the Paired t-test.

approximately 97% and 87%, respectively of that elicited by the caplet. The caplet elicited a significant 8% higher C_{max} than the gummy ($p = 0.031$), however, this is numerically small suggesting minimal clinical relevance. A sigmoidal relationship between dose and concentrations have been observed at doses <100 mg (8, 17). Similar to the pharmacokinetic profiles in plasma observed in this study, plasma concentrations reach a plateau between 70 and 80 umol/L when doses >100 mg are administered (17). Bioavailability is also inversely correlated with dose, with doses <100 mg reporting median bioavailability of ≥80%. Doses of 200, 500, and 1250 mg have been shown to have a median bioavailability of 72%, 63%, and 46%, respectively (17).

L-ascorbic acid is absorbed from the small intestine and achieves peak plasma concentration approximately 120–180 min after ingestion. It is then differentially absorbed by tissues and organs (11). L-ascorbic acid T_{max} was approximately 14 min faster for the gummy than the caplet, suggesting a non-significant, yet slightly quicker uptake of the gummy. In addition, the terminal half-life of the gummy was 87 min longer than the caplet suggesting a non-significant, yet relatively slower elimination of gummy than the caplet. Taken together, the plasma concentration of L-ascorbic acid vitafusion® Power C Gummy and a comparator caplet are similar, suggesting that absorption from the small intestine is not significantly different between the two formulations.

Intestinal absorption of L-ascorbic acid occurs through the sodium-dependent active transporter 1 (SVCT1) in a saturable and dose-dependent manner (18, 19). Efficacy claims focus on the uptake and incorporation into blood and tissue compartments (20). Previous studies have reported that food-derived, as well as supplement-derived vitamin C are equally bioavailable in humans (21). Biologically meaningful and clinically relevant studies evaluating the efficacy of vitamin C must account for the physiological concentrations achieved in blood and different tissue compartments. These physiological concentrations are dependent on absorption, distribution volume, cellular uptake, renal reabsorption and excretion (22). While the level of L-ascorbic acid in plasma reflects the amounts acutely absorbed from the digestive tract, its concentration in leukocytes is considered a better historical indicator of dietary intake, and absorption and retention in tissues. Therefore, studies that leukocyte as well as plasma uptake can better evaluate effective physiological concentrations. In this study, leukocyte total AUC_{0-24h}, $iAUC_{0-24h}$, and C_{max} were not significantly different between the gummy and caplet. The gummy elicited a significantly 115 min longer L-ascorbic acid T_{max} ($p = 0.012$) than the caplet. However, the total AUC_{0-24h}, $iAUC_{0-24h}$, and C_{max} elicited by the gummy were 92%, 103%, and 97%, respectively of that elicited by the caplet in leukocytes. In addition, there were no significant differences between the two groups in the urinary elimination of L-ascorbic acid. With increased intake, more rapid excretion occurs, with 30–50% and 56–80% of vitamin C excreted in urine in doses of 100 mg and 400 mg, respectively (8). As a high dose of vitamin C was used in this study, future studies examining lower doses of vitamin C may expect a lower rate of urinary elimination of L-ascorbic acid. Overall, the leukocyte concentration of L-ascorbic acid from vitafusion® Power C Gummy and a comparator caplet were similar, suggesting uptake into cells and tissues is not significantly different between the two formulations.

While this study examined the bioequivalence of two common delivery forms, gummies and caplets, future research should examine the bioavailability of other forms of vitamin C supplements include tablets and gel capsules. Moreover, it has been demonstrated that the menstrual cycle and cyclic changes in estradiol levels is correlated with

plasma vitamin C concentrations (23). Therefore, cyclic variation in plasma concentrations of L-ascorbic acid should be considered in future bioavailability studies.

Conclusion

In conclusion, both gummy and caplet exhibited similar absorption and bioavailability profiles with overlapping total AUC_{0-24h}, $iAUC_{0-24h}$, C_{max}, and T_{max} and were considered bioequivalent. This study therefore supports vitafusion® Power C Gummy as a viable alternative to the caplet for providing bioavailable vitamin C.

Acknowledgments

H. K. Z., W. H., A. W., and A. G. contributed to the inception and design of the study along with M. E., and N. G. All authors contributed to the writing and editing of the manuscript and approved the final version. We would also like to acknowledge the input of Mohammed Marzuk in the interpretation of urinary L-ascorbic acid amounts.

Declaration of conflict of interest

Church & Dwight Co., Inc., owns vitafusion® Power C Gummy and provided funding. H. Kelly Zhang, William Hooper, Andrew Wong, and Annahita Ghassemi are employees of Church & Dwight Co., Inc. These affiliations did not affect the interpretation of the results.

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