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Vitamin C-lipid metabolites: Uptake and retention and effect on plasma C-reactive protein and oxidized LDL levels in healthy volunteers

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: Previously, a novel formulation of vitamin C-lipid metabolites (PureWay-C®) was shown to be more rapidly taken-up by human T-lymphocytes and more rapidly stimulate neurite outgrowth, fibroblast adhesion and inhibition of xenobiotic-induced T-cell hyperactivation. Here, PureWay-C® serum levels were measured in healthy volunteers after oral supplementation. Plasma C-reactive protein and oxidized low density lipoprotein levels (LDL) were also measured.

Material/Methods: Healthy volunteers maintained a low vitamin C diet for 14 days and, following an overnight fast, received a single oral dose of (vitamin C) 1000 mg of either ascorbic acid (AA), calcium ascorbate (CaA), vitamin C-lipid metabolites (PureWay-C®), or calcium ascorbate-calcium threonate-dehydroascorbate (Ester-C®). Blood samples were collected immediately prior to the oral dose administration and at various times post ingestion. Twenty-four-hour urine collections were saved for oxalate and uric acid assays.

Results: PureWay-C® supplementation leads to the highest absolute serum vitamin C levels when compared to AA, CaA and Ester-C®. PureWay-C® provides a statistically significant greater serum level than calcium ascorbate at 1, 2, 4, and 6 hours post oral supplementation whereas Ester-C® shows a less but slightly statistically significant increase at only 1 and 4 hours. Oral supplementation with PureWay-C® also led to a greater reduction in plasma C-reactive protein and oxidized LDL levels compared to the other vitamin C formulations.

Conclusions: PureWay-C® is more rapidly absorbed and leads to higher serum vitamin C levels and greater reduction of plasma levels of inflammatory and oxidative stress markers than other forms of vitamin C, including Ester-C®.

key words: **vitamin C • lipid metabolites • absorption • serum levels • inflammation • oxidative stress**

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BACKGROUND

Vitamin C is a vital dietary component which is required for normal and healthy physiological and metabolic activities including neurite outgrowth, neuronal survival, wound healing events, and control of inflammation, [1–10]. These benefits of vitamin C in healthy physiological events have had obvious implications for a therapeutic value of vitamin C in the management of oxidative stress, management of inflammatory conditions, and recovery from stroke and tissue damage [11–17]. Recent research has attributed much of the beneficial effects of vitamin C supplementation on the reduction of circulating levels of inflammatory mediators and markers including C-reactive protein and oxidized low density lipoprotein (LDL) [11–13,15–17]. Therefore, improved vitamin C absorption rates and enhanced bioavailability of vitamin C in the body may have important clinical and life-style implications. Along these lines, a calcium ascorbate preparation with small amounts of dehydroascorbate, calcium threonate, xylonate and lyxonate, has been shown to lead to the increased cellular uptake of vitamin C [9,10], to provide increased protection from vitamin C deficiency in rats [18], and to improve uptake and circulating levels of vitamin C in humans [19].

In 2003, PureWay-C®, a proprietary formulation of vitamin C-lipid metabolites, was developed by Pedro Perez, Ph.D. at Innovation Laboratories, Inc. Recently, PureWay-C® has been shown to have strong antioxidant activity and to be more rapidly taken up by human T-lymphocytes than ascorbic acid, calcium ascorbate and calcium ascorbate-calcium threonate-dehydroascorbate (Ester-C®) [20]. Further, the increased rate of cellular absorption of PureWay-C® is associated with improved biological activity compared to ascorbic acid, calcium ascorbate and calcium ascorbate-calcium threonate-dehydroascorbate [1]. For example, PureWay-C® has been shown to most rapidly stimulate neurite formation, fibroblast adhesion and to provide protection to cell of the immune system from xenobiotic-induced hyperactivation [1]. The ability of PureWay-C® to more rapidly gain entry to cells and exert greater beneficial effects than ascorbic acid, calcium ascorbate, and calcium ascorbate-calcium threonate-dehydroascorbate may have great value to human health if this activity is also associated with an increased rate of absorption by the body after oral supplementation.

Here, healthy volunteers maintained a low vitamin C diet for 14 days; then, following an overnight fast, received a single oral dose of (vitamin C) 1000 mg of either ascorbic acid (AA), calcium ascorbate (CaA), vitamin C-lipid metabolites (PureWay-C®), or calcium ascorbate-calcium threonate-dehydroascorbate (Ester-C®). Blood samples were collected immediately prior to and after vitamin C intake and serum vitamin C levels were measured to determine if PureWay-C® was more rapidly taken-up in the body. In order to examine benefits of supplementation on circulating markers of inflammation, plasma C-reactive protein and oxidized LDL levels were also measured. Urine uric acid and oxalate were also measured to determine if there were any adverse effects.

MATERIAL AND METHODS

Materials

Formulations and certificates of analysis of ascorbic acid, calcium ascorbate, Ester-C® and PureWay-C® were provided by Nature's Value, Coram, NY, from their respective suppliers, and were dissolved in 118 ml of apple juice containing no significant vitamin C.

Study design and volunteers

This study was a prospective, randomized, double-blind trial studying four different vitamin C formulations on post-supplementation concentrations in serum as well as the effect on plasma biochemical markers of inflammation and oxidative stress. Forty healthy patients were randomly assigned into equal treatment groups (n=10), and received verbal and written information about how to reduce dietary vitamin C intake (a vitamin C-restricted diet) for a period of 14 days; on the night of the 14th day, the patients then fasted until the morning of the 15th day. A zero-hour blood sample was taken followed by the oral administration of a single test dose of 1000 mg of vitamin C (as ascorbic acid contained in the test product): PureWay-C® (10 patients); Ascorbic acid (10 patients); Calcium ascorbate (10 patients); and Ester-C® (10 patients). Then, post-treatment blood samples were taken at one, two, four, six and twenty-four hours. urine samples were taken and tested twenty-four hours past the oral treatment administration. All blood and urine samples were analyzed at the University of Miami School of Medicine (Miami, FL) and Esoterix Clinical Trial Services, a LabCorp Company (Research Triangle Park, NC).

Forty randomized healthy volunteers between the ages of 21 and 50 were recruited from the community and internal medicine outpatient clinics, and all provided informed consent prior to randomization. Volunteers were equally randomized in a blinded fashion to one of the four treatment groups. After the 14 days of a vitamin C-restricted diet followed by an overnight fasting, each patient was provided with the appropriate test product.

The inclusion criteria for the volunteers were as follows: Men or women between the ages of 21 and 50; adequate venous access; a normal history, a physical examination, laboratory tests, and a dental examination were required. The exclusion criteria were: congestive heart failure, uncontrolled arrhythmias, myocardial infarction, coronary bypass surgery, coronary angioplasty, or severe peripheral artery disease within the past 6 months, unstable angina pectoris, cigarette smoking, history of kidney stone, glucose-6-phosphate dehydrogenase deficiency, bleeding disorders, or family history of iron overload/hemochromatosis, inflammatory disease or taking anti-inflammatory drugs, uncontrolled endocrine or metabolic disorders known to influence serum lipids or lipoproteins, active or chronic hepatobiliary disease, ALT, AST >2.0 times the upper limit of normal, a baseline serum creatinine >2.5 mg/dL, fasting blood sugar >200 mg/d, poorly controlled (HbA1c >9%) or newly diagnosed diabetes mellitus, CK >2.0 times the upper limit of normal, diabetics recently started on oral hypoglycemic therapy (< 4weeks from randomization), baseline TSH level of >10mU/L, breast-feeding or pregnancy, childbearing age women without an effective method of contraception, cancer other than basal cell carcinoma within the past 5 years, and postmenopausal women on hormone replacement therapy.

All eligible, consenting volunteers were randomized at baseline and followed for 14 days of reduced dietary vitamin C intake (a vitamin C-restricted diet). The following data

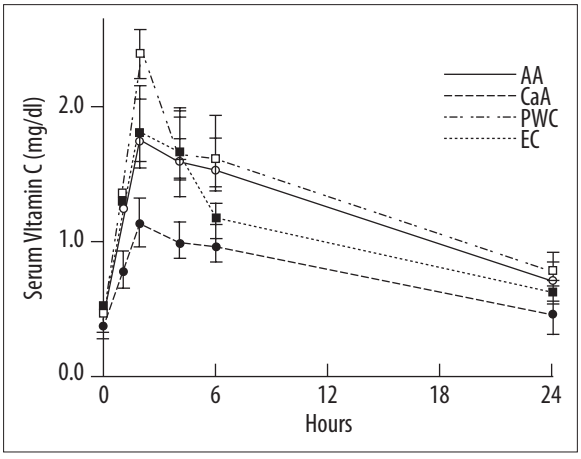


Figure 1. Serum vitamin C levels at various times post-supplementation. Volunteers were placed in groups of ten and supplemented with ascorbic acid (AA), calcium ascorbate (CaA), PureWay-C (PWC) or Ester-C (EC) and serum vitamin C concentrations were determined immediately prior (0 hours) and at hours 1, 2, 4, 6 and 24 post-supplementation as described in the materials and methods section. The data represent the mean + the S.E.M. Statistically different results were obtained between PWC and CaA at hours, 1, 2, 4, and 6 with p values of 0.0026, 0.0009, 0.0278 and 0.0470 respectively. EC also showed statistically significant differences from CaA at hours 1 and 4 with p values 0.049 and 0.0477 respectively. Confidence of statistical significance was determined using the paired T-test.

were collected at baseline, 0 hr (before-treatment), 1 hr, 2 hr, 4 hr, 6 hr and 24 hr (after-treatment): medical history, brief physical, weight, body mass index (BMI) and blood pressure, patient symptom checklist, fasting C-reactive protein, CBC, basic metabolic panel, TSH and β-HCG (baseline only); and for safety reasons, also creatinine, AST/ALT, CK and blood glucose, oxidized low-density lipoproteins, serum levels of ascorbic acid, plasma levels of C-reactive protein and oxidized low density lipoprotein, urine uric acid (at 24 hr only), and urine oxalate (at 24 hr only).

Measurement of serum, plasma and urine markers

Serum vitamin C levels were determined as previously described [21]. Briefly, blood was drawn in a chilled tube and immediately centrifuged to collect serum. The serum samples were frozen and protected from light. Vitamin C levels were then determined by high pressure liquid chromatography (HPLC) with electrochemical detection (EC).

Plasma C-reactive protein levels were measured according to the manufacturer's guidelines by instant ELISA from Bender MedSystems, Vienna, Austria.

Plasma levels of oxidized LDL was measured according to manufacturer's guidelines, using a solid phase two site oxidized LDL ELISA kit from Mercodia, Uppsala, Sweden, which is designed for use in measuring plasma oxidized LDL in arbitrary units (presented as U/ml in the results section.

Urine uric acid was measured by uricase as previously described [22] and urine oxalate levels were measured enzymatically using the Cobas Fara as previously described [23].

Results

The rate of vitamin C absorption in serum after oral administration was compared between several different vitamin C formulations. We found significantly different rates of uptake depending on the formulation of vitamin C. Prior to vitamin C supplementation the groups of ten healthy volunteers did not show a significant difference in serum vitamin C levels between or within groups. One hour after vitamin C administration, PureWay-C® showed the greatest serum absorption levels with a mean mg/dl concentration of 1.3 while Ester C showed a mean of 1.22 mg/dl and calcium ascorbate showed a mean of only 0.88 mg/dl (Figure 1). For both PureWay-C and Ester-C, this difference was statistically significant with p values of 0.0026 and 0.497 respectively. Two hours post administration, PureWay-C® again showed the highest absorption into serum at 2.17 mg/dl, which was statistically significantly higher than ascorbic acid (AA) at 1.64 mg/dl (p=0.05) and calcium ascorbate (CaA) at 1.12 mg/dl (p=0.009). PureWay-C® also showed statistically significantly higher levels than CaA at four hours (p=0.028) and six hours (p=0.047). Indeed, PureWay-C® demonstrated the highest serum vitamin C absorption levels at all times tested throughout the 24 hour period, including the 24 time point (Figure 1). In contrast, Ester-C failed to show a statistically significant increase in absorption when compared to all vitamin C formulation, with the exception of CaA at one hour (p=0.049) and four hours (p=0.047). All vitamin C formulations showed peak absorption levels at two hours post administration with only slightly elevated levels twenty-four hours post administration. However, it is worth noting that PureWay-C® maintains the highest serum level of 0.85 mg/dl vitamin C at 24 hours post treatment, which is nearly statistically significant compared to the 0.59 mg/ml observed with CaA at 24 hours (p=0.057). To a statistically significant extent, these data demonstrate that PureWay-C™ is better absorbed by the human body than Ascorbic Acid and Calcium Ascorbate, and that this increased absorption is greater than that observed with Ester-C.

In addition to the greatest absorption rates, PureWay-C® supplementation lead to the greatest drop in volunteer plasma C-reactive protein levels. While the drop in plasma C-reactive protein of 22.7 ng/ml was greater than that for AA (12 ng/ml), CaA (8.3 ng/ml) and Ester-C (20.6 ng/ml), the differences between these formulations was not statistically significant (Figure 2). Plasma oxidized low density lipoprotein levels (oxLDL) were also most greatly reduced by PureWay-C® compared to AA, CaA and Ester-C (Figure 3). PureWay-C® supplementation resulted in a 3.8U/ml drop in oxLDL which is a statistically significantly greater drop than that observed with AA (p=0.045) and again a greater and more beneficial effect that what was observed with CaA and Ester C.

Taken together, these data show that PureWay-C® is absorbed into the human body after oral supplementation to a greater extent than is Ester-C and that, once absorbed, it persists longer in the body, providing better health benefits with regard to circulating inflammatory markers. Urine uric acid and oxalate levels were not significantly elevated during this study, demonstrating that the increase activity of PureWay-C® is not associated with any of the adverse effects associated with mega doses of vitamin C.

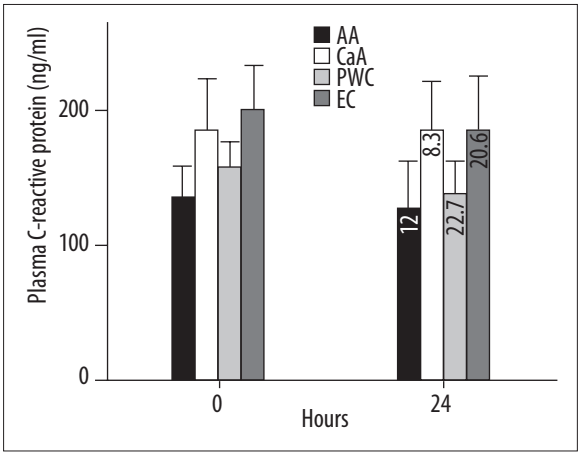


Figure 2. Plasma C-reactive protein levels before and after vitamin C supplementation. Volunteers were placed in groups of ten and supplemented with ascorbic acid (AA), calcium ascorbate (CaA), PureWay-C (PWC) or Ester-C (EC) and plasma C-reactive protein levels were determined immediately prior (0 hours) and 24 hour post-supplementation as described in the materials and methods section. The numbers in the bars at 24 hours are the change (decrease) in plasma C-reactive protein levels after vitamin C supplementation. These are the same data shown in Table 1, although the numbers shown for the change in C-reactive protein were rounded-off here to fit in the bar space.

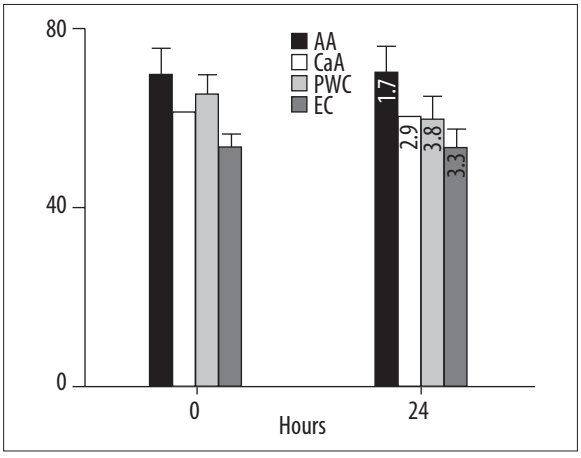


Figure 3. Plasma oxidized low density lipoprotein (oxLDL) levels before and after vitamin C supplementation. Volunteers were placed in groups of ten and supplemented with ascorbic acid (AA), calcium ascorbate (CaA), PureWay-C (PWC) or Ester-C (EC) and plasma oxLDL levels were determined immediately prior (0 hours) and 24 hour post-supplementation as described in the materials and methods section. The numbers in the bars at 24 hours are the change (decrease) in plasma oxLDL levels after vitamin C supplementation. These are the same data shown in Table 1, although the numbers shown for the change oxLDL were rounded-off here to fit in the bar space.

Table 1. Summary of the clinical data showing serum vitamin C levels, plasma C-reactive protein and oxidized LDL levels and urine uric acid and oxalate levels.

Serum Vitamin C Levels (mg/dl)						
	Hrs Post-Admin					
	0	1	2	4	6	24
Vitamin C Form						
Ascorbic Acid	0.56±0.06	1.2±0.10	1.64±0.18	1.51±0.22	1.46±0.13	0.80±0.09
Calcium Ascorbate	0.50±0.05	0.88±0.10	1.12±0.17	1.03±0.13	1.0±0.13	0.59±0.09
PureWay-C	0.56±0.09	1.3±0.08*	2.17±0.19*	1.54±0.14*	1.51±0.19*	0.85±0.08
Ester-C	0.60±0.08	1.22±0.11*	1.69±0.27	1.52±0.16*	1.17±0.12	0.73±0.07

	Plasma C-Reactive Protein (ng/ml)			Plasma OxLDL (U/ml)			Urine Markers (mg/dl)	
	0	24	Change	0	24	Change	Uric Acid	Oxalate
Ascorbic Acid	129.75±26	117.00±33	12.75	68.78±6	67.89±5	0.89	50.85±8.8	18.8±2.7
Calcium Ascorbate	189.17±41	180.83±43	8.34	60.56±5	57.67±6	3.78	39.75±10.5	17.8±2.6
PureWay-C	152.30±19	128.60±19	23.7	62.56±5	57.30±4	5.26**	48.73±7.1	13.7±1.5
Ester-C	200.63±38	180.00±52	20.63	50.51±4	48.20±4	2.31	40.96±7.0	17.9±1.9

***** Statistically significant deference compared to Calcium Ascorbate. At one hour p=0.0026 for PureWay-C and p=0.049 for Ester-C. At two hours, p=0.0009. At four hours p=0.0278 for PureWay-C and 0.0477 for Ester C. At six hours, p=0.0470; ****** Statistically significant difference from Ascorbic Acid (p=0.045). Note that the reductions in oxLDL were not significantly different for any vitamin C supplementation with a before-and-after comparison; however, the drop observed with PureWay-C was significantly greater than the drop observed with Ascorbic Acid; All statistically significant differences are noted. Data are presented as the mean + S.E.M. All 0 time points were immediately prior to oral administration of the vitamin C as described in the materials and methods section. These data are the same as those shown in Figures 1–3.

Discussion

Vitamin C is important in many physiological and metabolic activities such as the development of a healthy nervous system [1,2], prevention of neurodegenerative diseases [3,4], wound healing [5,6], and immune system function [1,7,8]. For these reasons, vitamin C supplementation has been recommended and people have sought forms of vita-

min C which lead to the fastest absorption into the blood and greatest health benefits.

Recently studies have suggested that vitamin C supplementation can reduce circulating levels of C-reactive protein and oxidized low density lipoproteins [11,12,15,17]. Oxidized LDLs and C-reactive proteins are produced and increased in the circulation during oxidative stress and have a subsequent role in the causation of inflammatory disease, atherosclerosis and cardiovascular damage [13,14,16]. Therefore, reduction of plasma C-reactive protein and oxidized LDL is not only an indicator of protection, but also will directly reduce risk for inflammatory damage and cardiovascular disease.

First developed and submitted for patent approval in 2003, 2006 and 2007 by Pedro Perez Ph.D, PureWay-C® has been shown to be absorbed more rapidly by human cells than any other vitamin C formulation tested, including Ester-C. Further, the more rapid absorption of PureWay-C® leads to a greater cellular activity in neurons, fibroblast and T-cells. The observations presented here show that PureWay-C® is also better absorbed into the human body after oral supplementation and results in higher serum vitamin C levels when compared to ascorbic acid, calcium ascorbate, and Ester-C. Further, PureWay-C® has a greater effect on circulating levels of C-reactive protein and oxidized LDL and while these data were not statistically significant, usually longer supplementation times of up to 2 months have been required to show significant drops in these oxidative stress markers [15–17]. Lastly, Pureway-C shows no adverse effects as judged by urine uric acid and oxalate levels.

Conclusions

Taken together, the data of this study show that PureWay-C® is statistically significantly better absorbed by the human body than ascorbic acid and calcium ascorbate at times (2 and 6 hours) post-supplementation which are times at which Ester-C shows no significant improved absorption. Further, PureWay-C shows a numerically better absorption mean that Ester-C at all time points, and although the difference is not highly statistically significant, the 2 hour and 6 hour increase of Pureway-C compared to Ester-C has p values of 0.137 and 0.162 respectively. Further, PureWay-C™ is the only Vitamin C formulation to show statistically significantly greater impact than ascorbic acid on circulating oxidized LDL levels. PureWay-C® supplementation also leads to the greatest reduction in circulating C-reactive protein. These results suggest that PureWay-C® is the best vitamin C formulation for supplementation with regard to cellular uptake and absorption into the human body. Future studies are needed with an increased population size and a longer supplementation regimen to examine if PureWay-C™ has improved effects on circulating C-reactive protein and oxidized LDL levels and to more clearly establish that PureWay-C® has improved health benefits when compared to other vitamin C formulations.

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