Comparative Bioavailability of Various Vitamin C Supplementation Forms

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1. Study Objective

To compare the bioavailability of various Vitamin C supplements in the liquid liposomal, powdered liposomal, and non-liposomal forms.

2. Methods

The current study was a four-group, randomized controlled trial of the effect of a single 1 000mg dose of two different formulations of liquid liposomal vitamin C (LLA and LLB), powdered liposomal vitamin C (PL), and non-liposomal vitamin C (NL).

2.1. Participants

Forty metabolically healthy volunteers were enrolled in the study. They were randomly and evenly assigned to one of the four supplementation groups. Participant exclusion criteria included:

- <20 and >50 years of age
- Any diagnosis of chronic condition(s)
- BMI outside of the normal category range (18.5-24.9kg/m^2)
- Presence of acute illness
- Use of drugs or dietary supplements on a frequent and/or mandatory basis

2.2.Active Substances/Supplementation groups

a. **Liposomal Product A (LLA)**
   Radiance Health Liposomal Vitamin C
   1 000mg in liquid form
   Manufactured in Hamburg, Germany
b. **Liquid Liposomal Product B (LLB)**
   1 000mg in liquid form
   Manufactured in The Netherlands
c. **Powdered Liposomal Product (PL)**
   1 000mg in powdered form
   Manufactured in the Netherlands
d. **Non-Liposomal Product (NL)**
   1 000mg in tablet form
   Manufactured in the UK

2.3. Dosage and Blood Collection

An oral dose of 1 000mg of Vitamin C for the respective supplement groups was administered to fasted participants. Blood was collected in each group at baseline (before supplement administration), 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 16 hours, 20 hours, and 24 hours after supplement administration. Blood was microcentrifuged, cooled to 2°C, and analyzed for plasma Ascorbic Acid (AA) levels via High-Performance Liquid Chromatography (HPLC) at the laboratories of Surya Research Clinics.

2.4. Statistics

The pharmacokinetic parameters max concentration (C_{max}) and time to max concentration (T_{max}) were taken from the raw data. Area under the curve from baseline to the final measurement (AUC_{0-t}) was calculated using the trapezoidal rule. Incremental AUC ($iAUC_{0-t}$) was calculated using the trapezoidal rule to account for the baseline levels of AA present in plasma. Significant differences between AUC and iAUC between each group were measured with an ANOVA. Oral bioavailability value (OBV) was calculated using $iAUC_{0-t}$ to compare the bioavailability of all possible combinations of supplement forms.

A two-way ANOVA examined the effect of supplement type and time of blood draw on plasma AA levels between all possible combinations of the study arms. A repeated measured ANOVA examined the effect of supplementation over time in each individual group. Statistical significance was set at p=0.05. All analyses were conducted using the statistical software Jamovi 1.2.17, unless otherwise stated.

3. Results

All enrolled participants completed the study. Participants were in their late twenties, equally split between male and female, and had a healthy BMI and blood pressure. Participant anthropometric data are presented in *Table 1.*

Table 1. Participant Anthropometric Data

	LLA[ab]	LLB[ab]	PL[ab]	NL[ab]
Age (years)	24,40 (5,10)	26,10 (4,90)	27,20 (6,20)	28,30 (6,60)
Females (%)	50	40	60	40
BMI (kg/m^2)	20,40 (1,50)	20,90 (1,60)	20,50 (1,40)	20,20 (1,60)
Systolic BP (mmHg)	119,60 (12,20)	118,40 (12,90)	117,30 (10,40)	118,60 (12,60)
Diastolic BP (mmHg)	78,20 (8,60)	77,70 (7,80)	80,10 (9,20)	78,40 (6,80)

[a] Mean (SD). [b] n=10

3.1. Pharmacokinetics

C_{max} was 2.78 mg/dL in the LLA group and was reached at a T_{max} of 12 hours. C_{max} was 2.51 mg/dL in the LLB group and was reached at a T_{max} of 8 hours. C_{max} was 0.97 mg/dL in the PL group and was reached at a T_{max} of 4 hours. C_{max} was 1.12mg/dL in the NL group and was reached at a T_{max} of 4 hours.

The LLA group had an AUC_{0-t} of 51.86 mg*hr/dL and an $iAUC_{0-t}$ of 36.76 mg*hr/dL. The LLB group had an AUC_{0-t} of 34.51 mg*hr/dL and an $iAUC_{0-t}$ of 19.15 mg*hr/dL. The PL group had an AUC_{0-t} of 15.19 mg*hr/dL and an $iAUC_{0-t}$ of 1.70 mg*hr/dL. The NL group had an AUC_{0-t} of 17.61 mg*hr/dL and an $iAUC_{0-t}$ of 3.02 mg*hr/dL. Pharmacokinetic data are presented in *Table 2.* A graphical representation of plasma AA levels in each group over time is shown in *Figure 1.*

These values resulted in the LLA group being 1.92 times more bioavailable as the LLB group, 21.64 times more bioavailable as the PL group and 12.17 times more bioavailable as the NL group.

Table 2. Vitamin C Pharmacokinetic Parameters

Measurements	LLA	LLB	PL	NL
C_{max} (mg/dL)	2,78	2,51	0,97	1,12
T_{max} (hours)	12	8	4	4
AUC_{0-t} (mg*hr/dL)[a]	51,86	34,51	15,19*	17,61*
$iAUC_{0-t}$ (mg*hr/dL)[a]	36,76	19,15	1,70*	3,02*

[a] AUC and iAUC calculated using trapezoidal rule
* Value is significantly different from group LLA

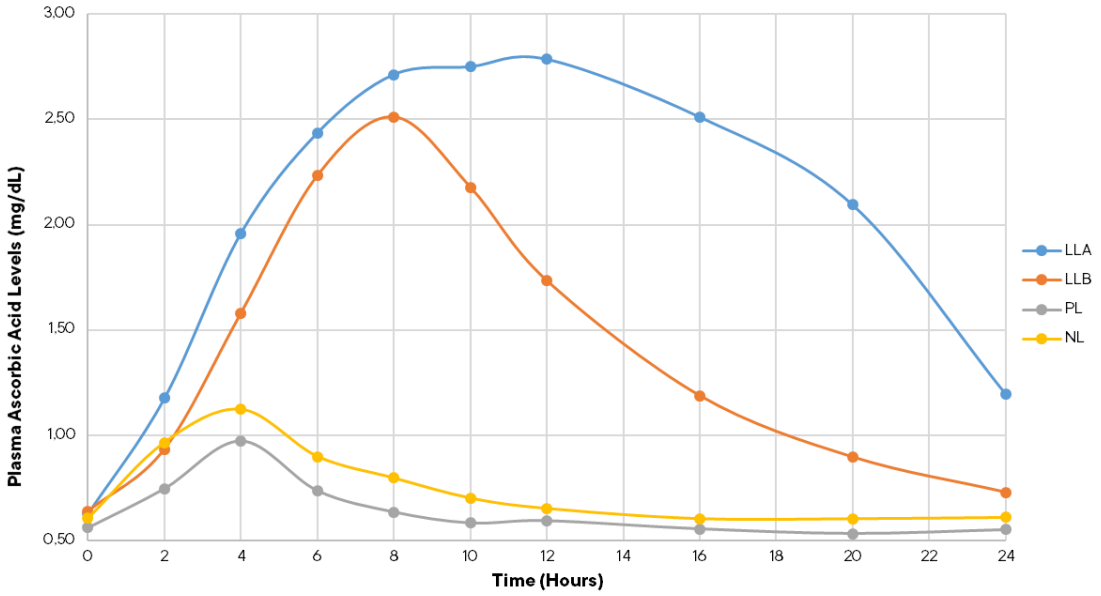

Figure 1. *Plasma AA levels over time after administration of 1 000mg Vitamin C in various supplementation forms.*

3.2. Between Group Analysis

3.2.1. *LLA vs. LLB*

The iAUC values showed that the LLA group was 1.92 times more bioavailable than the LLB group. However, no significant differences were found between the AUC and iAUC values (p=0.101 and p=0.094, respectively). There was a statistically significant interaction between the treatment and time of blood draw on the plasma AA levels between these groups, F (9.180) =9,28, p=<0.001. A Tukey post hoc test showed that the LLA group had significantly higher levels of plasma AA levels at 10 hours (p=0.032), 12 hours (p=<0.001), 16 hours (p=<0.001), and 20 hours (p=<0.001) when compared to the LLB group. There were no significant differences between groups in the remaining time points. These results are presented in *Table 3* and *Figure 2*.

Table 3. Between-Group Change in Mean Plasma AA Levels in LLA vs. LLB Supplement Groups[a]

Time Point	LLA[bcd]	LLB[bcd]	Difference of Means[c]	P-Value
Baseline	0,63 (0,04)	0,64 (0,03)	-0,01 (0,15)	1,000
2 Hours	1,18 (0,09)	0,94 (0,08)	0,24 (0,15)	0,989
4 Hours	1,96 (0,07)	1,58 (0,10)	0,38 (0,15)	0,596
6 Hours	2,44 (0,10)	2,23 (0,10)	0,20 (0,15)	0,999
8 Hours	2,71 (0,15)	2,51 (0,11)	0,20 (0,15)	0,999
10 Hours	2,75 (0,14)	2,18 (0,15)	0,57 (0,15)	0,032*
12 Hours	2,78 (0,12)	1,73 (0,14)	1,05 (0,15)	<0,001*
16 Hours	2,51 (0,15)	1,19 (0,08)	1,32 (0,15)	<0,001*
20 Hours	2,09 (0,16)	0,90 (0,06)	1,20 (0,15)	<0,001*
24 Hours	1,20 (0,11)	0,73 (0,04)	0,47 (0,15)	0,212

[a] Data analyzed using two-way ANOVA with Tukey post hoc test. [b] n=10. [c] Mean (SE). [d] Unit mg/dL.
* P-Value <0.05 is statistically significant

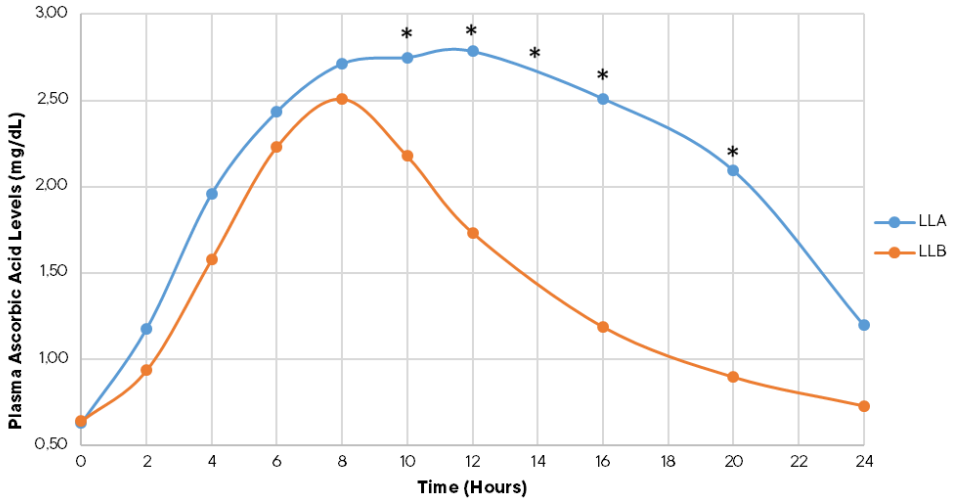

Figure 2. *Plasma AA levels over time after a single dose of 1 000mg of vitamin C in the LLA vs. LLB supplement group.*
**Indicates a significant difference between groups.*

LLA vs. PL

The iAUC values showed that the LLA group was 21.64 times more bioavailable than the PL group. Both the AUC and iAUC values of the LLA group were significantly higher than PL group (p=<0.001 and p=<0.001, respectively). There was a statistically significant interaction between the treatment and time of blood draw on the plasma AA levels between these groups, F (9, 179) = 39.4, p=<0.001. A Tukey post hoc test showed that the LLA group had significantly higher levels of plasma AA levels at every time point from hour 4 to hour 24 (p=<0.001) when compared to the PL group. There were no significant differences between groups in the remaining time points. These results are presented in *Table 4* and *Figure 3*.

Table 4. Between-Group Change in Mean Plasma AA Levels in LLA vs. PL Supplement Groups[a]

Time Point	LLA[bcd]	PL[bcd]	Difference of Means[c]	P-Value
Baseline	0,63 (0,04)	0,56 (0,54)	0,07 (0,13)	1,000
2 Hours	1,18 (0,09)	0,78 (0,75)	0,43 (0,13)	0,079
4 Hours	1,96 (0,07)	0,97 (0,97)	0,99 (0,13)	<0,001
6 Hours	2,44 (0,10)	0,74 (0,72)	1,70 (0,13)	<0,001
8 Hours	2,71 (0,15)	0,64 (0,61)	2,08 (0,13)	<0,001
10 Hours	2,75 (0,14)	0,59 (0,55)	2,16 (0,13)	<0,001
12 Hours	2,78 (0,12)	0,60 (0,61)	2,19 (0,13)	<0,001
16 Hours	2,51 (0,15)	0,56 (0,53)	1,95 (0,13)	<0,001
20 Hours	2,09 (0,16)	0,54 (0,50)	1,56 (0,13)	<0,001
24 Hours	1,20 (0,11)	0,55 (0,52)	0,65 (0,13)	<0,001

[a] Data analyzed using two-way ANOVA with Tukey post hoc test. [b] n=10. [c] Mean (SE). [d] Unit mg/dL.
* P-Value <0.05 is statistically significant

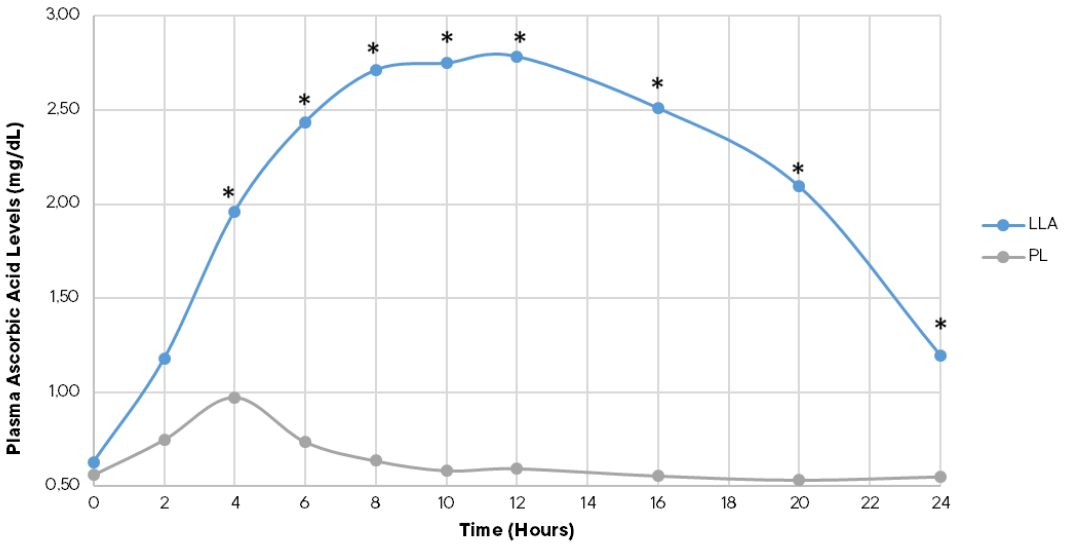

Figure 3. *Plasma AA levels over time after a single dose of 1 000mg of vitamin C in the LLA vs. PL supplement group.*
**Indicates a significant difference between groups.*

LLA vs. NL

The iAUC values showed that the LLA group was 12.17 times more bioavailable than the NL group. Both the AUC and iAUC values of the LLA group were significantly higher than NL group (p=<0.001 and p=<0.001, respectively). There was a statistically significant interaction between the treatment and time of blood draw on the plasma AA levels between these groups, F (9, 180) =39.1, p=<0.001. A Tukey post hoc test showed that the LLA group had significantly higher levels of plasma AA levels at every time point from hour 4 to hour 24 (p=<0.001) when compared to the NL group. There were no significant differences between groups in the remaining time points. These results are presented in *Table 5* and *Figure 4*.

Table 5. Between-Group Change in Mean Plasma AA Levels in LLA vs. NL Supplement Groups[a]

Time Point	LLA[bcd]	NL[bcd]	Difference of Means[c]	P-Value
Baseline	0,63 (0,04)	0,61 (0,05)	0,02 (0,13)	1,000
2 Hours	1,18 (0,09)	0,97 (0,07)	0,21 (0,13)	0,982
4 Hours	1,96 (0,07)	1,12 (0,06)	0,84 (0,13)	<0,001*
6 Hours	2,44 (0,10)	0,90 (0,04)	1,54 (0,13)	<0,001*
8 Hours	2,71 (0,15)	0,80 (0,03)	1,91 (0,13)	<0,001*
10 Hours	2,75 (0,14)	0,70 (0,02)	2,05 (0,13)	<0,001*
12 Hours	2,78 (0,12)	0,65 (0,02)	2,13 (0,13)	<0,001*
16 Hours	2,51 (0,15)	0,61 (0,02)	1,91 (0,13)	<0,001*
20 Hours	2,09 (0,16)	0,61 (0,04)	1,49 (0,13)	<0,001*
24 Hours	1,20 (0,11)	0,61 (0,05)	0,59 (0,13)	0,001*

[a] Data analyzed using two-way ANOVA with Tukey post hoc test. [b] n=10. [c] Mean (SE). [d] Unit mg/dL

* P-Value <0.05 is statistically significant

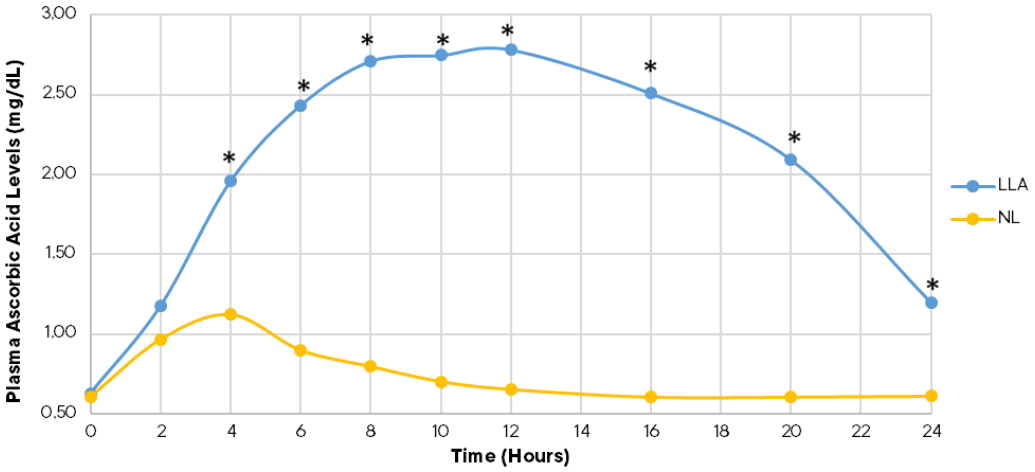

Figure 4. *Plasma AA levels over time after a single dose of 1 000mg of vitamin C in the LLA vs. NL supplement group.*

**Indicates a significant difference between groups.*

LLB vs. PL

The iAUC values showed that the LLB group was 11.26 times more bioavailable than the PL group. Both the AUC and iAUC values of the LLB group were significantly higher than PL group (p=0.002 and p=0.003, respectively). There was a statistically significant interaction between the treatment and time of blood draw on the plasma AA levels between these groups, F (9, 179) 41.8, p=<0.001. A Tukey post hoc test showed that the LLB group had significantly higher levels of plasma AA levels at every time point from hour 4 to hour 16 (p=<0.001) when compared to the PL group. There were no significant differences between groups in the remaining time points. These results are presented in *Table 6* and *Figure 5.*

Table 6. Between-Group Change in Mean Plasma AA Levels in LLB vs. PL Supplement Groups[a]

Time Point	LLB[bcd]	PL[bcd]	Difference of Means[c]	P-Value
Baseline	0,64 (0,03)	0,56 (0,54)	0,08 (0,10)	1,000
2 Hours	0,94 (0,08)	0,78 (0,75)	0,19 (0,10)	0,952
4 Hours	1,58 (0,10)	0,97 (0,97)	0,61 (0,10)	<0,001*
6 Hours	2,23 (0,10)	0,74 (0,72)	1,50 (0,10)	<0,001*
8 Hours	2,51 (0,11)	0,64 (0,61)	1,87 (0,10)	<0,001*
10 Hours	2,18 (0,15)	0,59 (0,55)	1,59 (0,10)	<0,001*
12 Hours	1,73 (0,14)	0,60 (0,61)	1,14 (0,11)	<0,001*
16 Hours	1,19 (0,08)	0,56 (0,53)	0,63 (0,10)	<0,001*
20 Hours	0,90 (0,06)	0,54 (0,50)	0,36 (0,10)	0,058
24 Hours	0,73 (0,04)	0,55 (0,52)	0,18 (0,10)	0,972

[a] Data analyzed using two-way ANOVA with Tukey post hoc test, [b] n=10, [c] Mean (SE), [d] Unit mg/dL.
* P-Value <0.05 is statistically significant

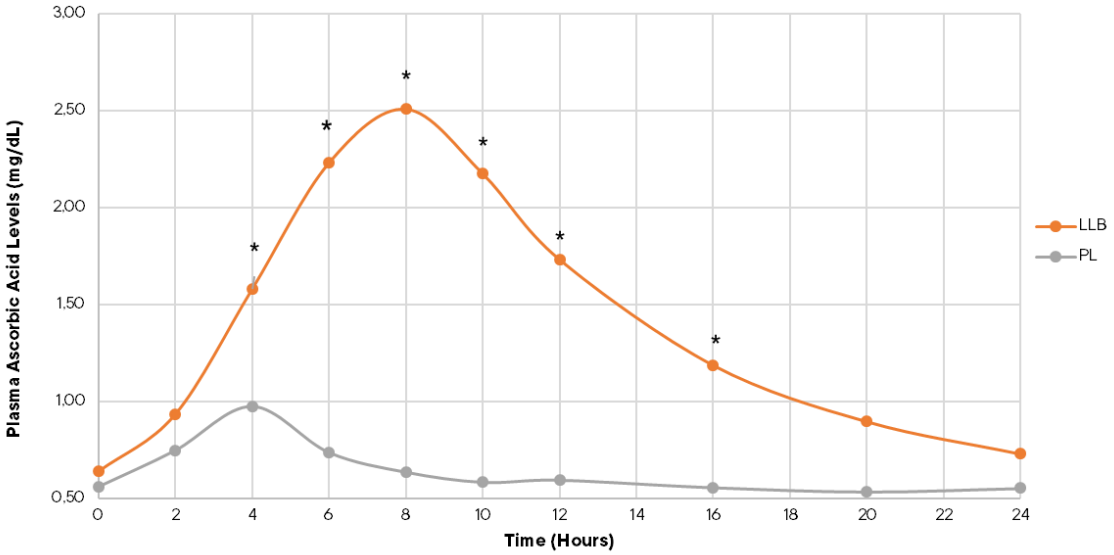

Figure 5. *Plasma AA levels over time after a single dose of 1 000mg of vitamin C in the LLvs. PL supplement group.*

**Indicates a significant difference between groups.*

LLB vs. NL

The iAUC values showed that the LLB group was 6.34 times more bioavailable than the NL group. There was no significant difference between AUC (p=0.276), but there was a significant difference in iAUC (p=0.007). There was a statistically significant interaction between the treatment and time of blood draw on the plasma AA levels between these groups, F (9, 180) =38.1. P=<0.001. A Tukey post hoc test showed that the LLB group had significantly higher levels of plasma AA levels at every time point from hour 4 to hour 16 (p=<0.001) when compared to the NL group. There were no significant differences between groups in the remaining time points. These results are presented in *Table 7* and *Figure 6.*

Table 7. Between-Group Change in Mean Plasma AA Levels in LLB vs. NL Supplement Groups[a]

Time Point	LLB[bcd]	NL[bcd]	Difference of Means[c]	P-Value
Baseline	0,64 (0,03)	0,61 (0,05)	0,03 (0,10)	1,000
2 Hours	0,94 (0,08)	0,97 (0,07)	-0,03 (0,10)	1,000
4 Hours	1,58 (0,10)	1,12 (0,06)	0,46 (0,10)	0,003*
6 Hours	2,23 (0,10)	0,90 (0,04)	1,33 (0,10)	<0,001*
8 Hours	2,51 (0,11)	0,80 (0,03)	1,71 (0,10)	<0,001*
10 Hours	2,18 (0,15)	0,70 (0,02)	1,15 (0,10)	<0,001*
12 Hours	1,73 (0,14)	0,65 (0,02)	1,08 (0,10)	<0,001*
16 Hours	1,19 (0,08)	0,61 (0,02)	0,58 0,10)	<0,001*
20 Hours	0,90 (0,06)	0,61 (0,04)	0,29 (0,10)	0,364
24 Hours	0,73 (0,04)	0,61 (0,05)	0,12 (0,10)	1,000

[a] Data analyzed using two-way ANOVA with Tukey post hoc test. [b] n=10. [c] Mean (SE). [d] Unit mg/dL.
* P-Value <0.05 is statistically significant

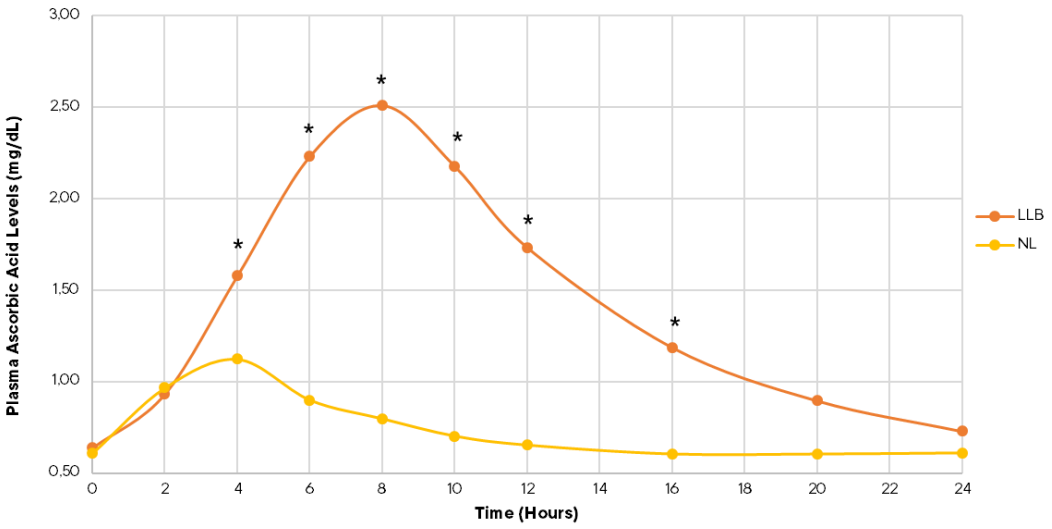

Figure 6. *Plasma AA levels over time after a single dose of 1 000mg of vitamin C in LLB vs. NL supplement form.*

**Indicates a significant difference between groups.*

PL vs. NL

The iAUC values showed that the NL group was 1.78 times more bioavailable than the PL group. No significant differences were found between the AUC and iAUC values (p=0.179 and p=0.352, respectively). There was no statistically significant interaction between the treatment and time of blood draw on the plasma AA levels between these groups, F (9, 179) =1.26, p=0.260. These results are not presented.

3.3. Within-Group Analysis

Data was also analyzed within each group to determine changes in plasma AA levels from baseline. Results showed that the LLA group achieved significantly higher plasma AA levels after hour 2 and maintained significantly higher levels through hour 24. The LLB group achieved significantly higher plasma levels when compared to baseline from hour 4 to hour 16. The PL and NL group achieved significantly higher plasma levels when compared to baseline from hour 2 through hour 6.

4. Discussion and Conclusions

Vitamin C is an essential nutrient with a variety of functions in the body. Vitamin C can maintain normal immune system function, contribute to normal metabolism and psychological function, protect the cells from oxidative stress and reduce tiredness and fatigue.[1] Bioavailability of vitamin C is limited, especially at high doses.[1] Liposomes have been shown to increase the bioavailability of various nutrients, including vitamin C, and have become popular in both the liquid and powdered form.[2] The present study was conducted to assess the bioavailability of this essential nutrient in various liposomal supplementation forms and to compare these forms to a traditional, non-liposomal vitamin C product.

Overall, the Radiance Health Liposomal Vitamin C supplement (LLA) had the highest bioavailability compared to the other supplement groups. LLA was 1.92 times more bioavailable than the competitor liquid liposomal product (LLB), 21.64 times more bioavailable than the powdered liposomal product (PL), and 12.17 times more bioavailable than the non-liposomal product (NL).

LLA achieved the highest C_{max}, AUC, and iAUC compared to the other supplement groups. Although the AUC and iAUC were not significantly different between LLA and LLB, LLA achieved a higher C_{max}. In addition, LLA had significantly higher C_{max}, AUC and iAUC values than the PL and NL groups. These results show that the LLA supplement achieved the highest plasma AA levels and maintained elevated levels for the longest period of time, resulting in an overall greater exposure to vitamin C than any other group.

This increased exposure within the LLA group can also be seen in the within group data analysis. Results showed that the LLA group was able to maintain plasma AA levels higher than baseline for the entire duration of the study, 22 hours of exposure. However, the LLB group only exhibited significant increases for 8 hours and the PL and NL groups only for 4 hours. These data show the strong impact liquid liposomes can have on overall exposure to vitamin C and the superiority of Radiance Health's product.

The differences between the two liquid liposomal products may be explained by varying production methods and resulting liposome quality. Production standards impact important liposome properties, such as size and encapsulation efficiency, that can then impact their effectiveness at delivering the active ingredient to the blood stream. These factors were not measured in the present study, but potential differences in production methods could possibly explain differences seen between the two liquid liposomal groups, assuming a higher quality liposome in the LLA group.

The PL supplement had the lowest C_{max} and AUC compared to the other supplement groups. Powdered liposomes are produced and marketed as a more shelf-stable and user-friendly version of liposomal products. They also maintain the claims for increased bioavailability. However, we see here, that the powdered liposomal product performed only as well as the non-liposomal product. The manufacturing of powdered liposomes can sometimes disrupt the structure and integrity of the liposome due to the lack of water. The disruption of the liposomal structure from the manufacturing process may be one explanation as to why this product performed poorly.

Overall, we see that liquid liposomal supplements significantly increased the bioavailability of vitamin C when compared to powdered or non-liposomal products.

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