Bioavailability of a Liposomal Vitamin D3K2 Product

August 2020

1. Study Objective

To evaluate the bioavailability of a liposomal vitamin D3K2 supplement (liposomal) compared to a non-liposomal vitamin D3K2 supplement (standard).

2. Methods

The current study was a two-group, randomized controlled trial of the effect of a liposomal versus standard vitamin D3K2 supplement on serum 25-hydroxyvitamin D (25(OH)D) and plasma K2-MK7 levels measured for six hours after administration of a single dose of a combined product.

2.1. Participants

Twenty metabolically healthy participants enrolled in the study. They were randomly and evenly assigned to either the liposomal or standard supplementation group. 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

a. **Liposomal Product:**
Purazell Liposomal Vitamin D3K2
Company:
Purazell GmbH
Monheim am Rhein, Germany

b. **Standard Product:**
Pinnacle Nutrition Vitamin D3K2 Tablets
London, United Kingdom

2.3. Dosage and Blood Collection

An oral dose of 1000 IUs of vitamin D3 and 200 µg of vitamin K2-MK7 from the combined liposomal or standard supplement was administered to fasted participants. Blood was collected at baseline (B0), and at 120 minutes (T1), 240 minutes (T2), and 360 minutes (T3) after administration. Blood was microcentrifuged for 12 minutes, cooled at 4°C and measured for serum 25(OH)D and plasma K2-MK7 levels using Liquid Chromatography-Tandem Mass Spectrophotometry (LC-APCI-MS/MS) techniques at the laboratories of Surya Research Clinics.

2.4. Statistics

Pharmacokinetic parameters of max concentration (C_{max}) and time to max concentration (T_{max}) are presented. Area under the curve from baseline to the final measurement (AUC_{0-t}) was calculated using the trapezoidal rule and oral bioavailability value (OBV) was determined as liposomal AUC_{0-t} /standard AUC_{0-t}.

A two-way ANOVA was conducted that examined the effect of supplement type and time of blood draw on serum 25(OH)D levels as well as plasma K2-MK7 levels. Significant differences were found using Tukey's comparison of means.

A one-way repeated measures ANOVA was conducted to examine the effects of time on supplementation within both the liposomal and standard groups for both serum 25(OH)D levels and plasma K2-MK7 levels. Significant effects of time on each group were determined using Tukey's pairwise comparisons. All analyses were conducted using the statistical software Jamovi 1.2.17.

3. Results

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

3.1. Vitamin D3

C_{max} was 52.94 ng/mL in the liposomal group and was reached at a T_{max} of 360 minutes. C_{max} was 25.60 ng/mL in the standard group and was reached at a T_{max} of 240 minutes. AUC_{0-t} was 140.49 ng*hr/mL in the liposomal group and 72.87 ng*hr/mL in the standard group resulting in an OBV of 1.93. Pharmacokinetic data are presented in *Table 2.* A graphical representation of serum 25(OH)D levels over time is shown in *Figure 1.*

There was a statistically significant interaction between the treatment and time of blood draw on the serum 25(OH)D levels, F (3,72) = 8.02, p=<0.001. Tukey's comparison of means showed that the liposomal group had significantly higher levels of serum 25(OH)D levels at T1 (p=<0.001), T2 (p=<0.001), and T3 (p=<0.001) when compared to the standard group. There were no significant differences between groups at B0 (p=0.981). These results are presented in Table 3.

There was a statistically significant effect of time on serum 25(OH)D levels in the liposomal group, F (3,27) = 27.60, p=<0.001. Tukey's pairwise comparisons revealed a significant increase in serum 25(OH)D levels from, B0-T1 (p=<0.001), B0-T2 (p=<0.001), and B0-T3 (p=<0.001). There were no significant differences between serum 25(OH)D levels from T1-T2 (p=0.834), T1-T3 (p=0.639), and T2-T3 (p=0.985). These results are presented in Table 4.

There was a statistically significant effect of time in the standard group, F (3,27) = 8.59, p=<0.001. There was a significant increase from B0 to T2 (p=<0.001), T1-T2 (p=0.043), and T2-T3 (p=<0.024). There were no significant differences between the remaining time points. These data are presented in Table 5.

Table 1. Participant Anthropometric Data

	Liposomal[ab]	Standard[ab]
Age (years)	25.60 (4.00)	27.10 (3.80)
Females (%)	40	30
BMI (kg/m2)	20.90 (1.70)	21.60 (1.60)
Systolic BP (mmHg)	118.20 (15.00)	120.10 (14.10)
Diastolic BP (mmHg)	74.60 (8.40)	76.30 (9.20)

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

Table 2. Vitamin D3 Pharmacokinetic Parameters

	Liposomal	Standard
C_{max} (ng/mL)	52.94	25.60
T_{max} (minutes)	360	240
AUC_{0-t} (ng*hr/mL)	140.49	72.87
OBV	1.93	

Table 3. Between-Group Change in Mean Serum 25(OH)D Levels[a]

Time Point	Liposomal[bcd]	Standard[bcd]	Difference of Means[cd]	P-Value
B0	26.53 (1.32)	22.71 (1.26)	3.82 (4.09)	0.981
T1	48.96 (3.67)	23.99 (0.94)	24.97 (4.09)	<0.001*
T2	51.78 (4.61)	25.60 (1.24)	26.18 (4.09)	<0.001*
T3	52.94 (5.01)	23.84 (1.13)	29.10 (4.09)	<0.001*

[a] Data analyzed using two-way ANOVA with Tukey's Comparison of Means, [b] n=10, [c] Mean (SE), [d] Unit ng/mL
* P-Value <0.05 is statistically significant

Table 4. Within-Group Change in Mean Serum 25(OH)D Levels in Liposomal Group[ab]

Time Point	Difference of Means[cd]	P-Value
B0-T1	22.40 (3.35)	<0.001*
B0-T2	25.22 (3.35)	<0.001*
B0-T3	26.38 (3.35)	<0.001*
T1-T2	2.82 (3.35)	0.834
T1-T3	3.98 (3.35)	0.639
T2-T3	1.16 (3.35)	0.985

[a] Data analyzed using one-way repeated measures ANOVA with Tukey's pairwise comparisons, [b] n=10, [c] Mean (SE), [d] Unit ng/mL
* P-Value <0.05 is statistically significant

Table 5. Within-Group Change in Mean Serum 25(OH)D Levels in Standard Group[ab]

Time Point	Difference of Means[cd]	P-Value
B0-T1	1.28 (0.57)	0.141
B0-T2	2.89 (0.57)	<0.001*
B0-T3	1.13 (0.57)	0.225
T1-T2	1.61 (0.57)	0.043*
T1-T3	0.15 (0.57)	0.994
T2-T3	-1.76 (0.57)	0.024*

[a] Data analyzed using one-way repeated measures ANOVA with Tukey's pairwise comparisons, [b] n=10, [c] Mean (SE), [d] Unit ng/mL
* P-Value <0.05 is statistically significant

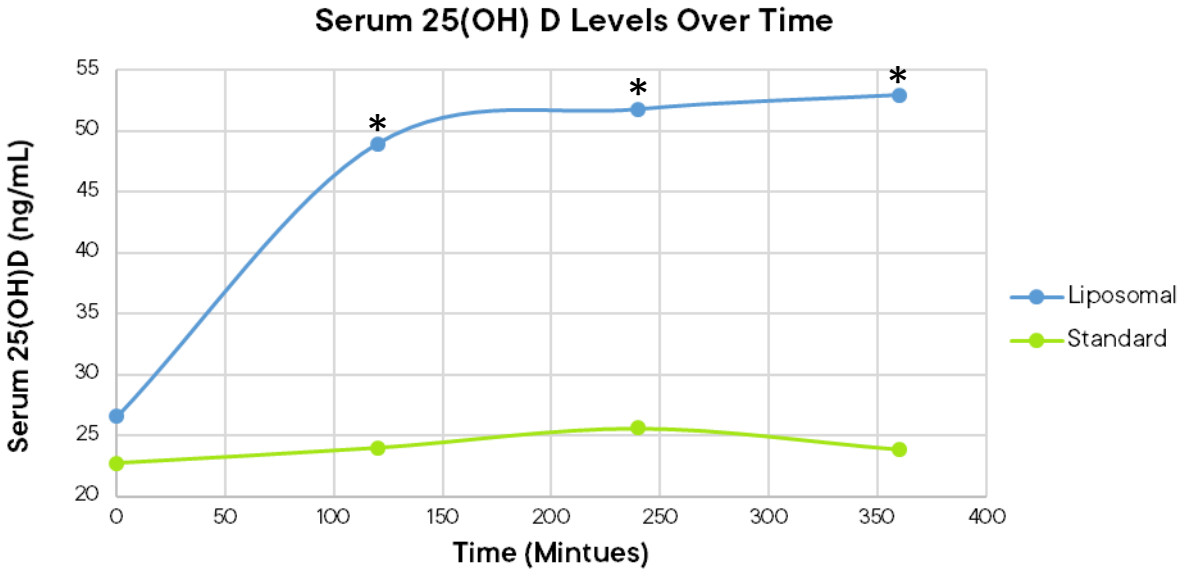

Figure 1. Serum 25-Hydroxyvitamin D levels over time after administration of 1000IU Vitamin D3 supplement in liposomal or standard form
* Indicates a significant difference between groups at the given time point (p<0.05)

3.2. Vitamin K2-MK7

C_{max} was 4.26 ng/mL in the liposomal group and was reached at a T_{max} of 240 minutes. C_{max} was 2.39 ng/mL in the standard group and was reached at a T_{max} of 240 minutes. AUC_{0-t} was 11.37 ng*hr/mL in the liposomal group and 6.96 ng*hr/mL in the standard group resulting in an OBV of 1.63. Pharmacokinetic data are presented in *Table 6.* A graphical representation of plasma K2-MK7 levels over time is shown in *Figure 2.*

There was a statistically significant interaction between the treatment and time of blood draw on plasma K2-MK7 levels, F (3,71) = 5.85, p=0.001. Tukey's comparison of means showed that the liposomal group had significantly higher levels of plasma K2-MK7 levels at T1 (p=0.002), T2 (p=<0.001), and T3 (p=<0.001) when compared to the standard group. There were no significant differences between groups at B0 (p=1.00). These data are presented in *Table 7.*

There was a statistically significant effect of time on plasma K2-MK7 levels in the liposomal group, F (3,27) = 17.00, p=<0.001. Tukey's pairwise comparisons revealed a significant increase in plasma K2-MK7 levels from, B0-T1 (p=<0.001), B0-T2 (p=<0.001), and B0-T3 (p=<0.001). There were no significant differences between plasma K2-MK7 levels from T1-T2 (p=0.461), T1-T3 (p=0.461), and T2-T3 (p=1.00). These results are presented in *Table 8.* No significant change in plasma K2-MK7 levels was seen over time in the standard group F (3,24) = 1.50, p=0.240.

Table 6. Vitamin K2 Pharmacokinetic Parameters

	Liposomal	Standard
C_{max} (ng/mL)	4.26	2.39
T_{max} (minutes)	240	240
AUC_{0-t} (ng*hr/mL)	11.37	6.96
OBV	1.63	

Table 7. Between-Group Change in Mean Plasma K2-MK7 Levels[a]

Time Point	Liposomal[bcd]	Standard[bcd]	Difference of Means[cd]	P-Value
B0	2.35 (0.09)	2.28 (0.11)	0.07 (0.36)	1.00
T1	3.80 (0.43)	2.26 (0.12)	1.54 (0.36)	0.002*
T2	4.26 (0.39)	2.39 (0.08)	1.87 (0.36)	<0.001*
T3	4.26 (0.35)	2.33 (0.09)	1.93 (0.37)	<0.001*

[a] Data analyzed using two-way ANOVA with Tukey's Comparison of Means, [b] n=10, [c] Mean (SE), [d] Unit ng/mL

* P-Value <0.05 is statistically significant

Table 8. Within-Group Change in Mean Plasma K2-MK7 Levels in Liposomal Group[ab]

Time Point	Difference of Means[cd]	P-Value
B0-T1	1.45 (0.31)	<0.001
B0-T2	1.91 (0.31)	<0.001
B0-T3	1.91 (0.31)	<0.001
T1-T2	0.46 (0.31)	0.461
T1-T3	0.46 (0.31)	0.461
T2-T3	0.00 (0.31)	1.000

[a] Data analyzed using one-way repeated measures ANOVA with Tukey's pairwise comparisons, [b] n=10, [c] Mean (SE), [d] Unit ng/mL
* P-Value <0.05 is statistically significant

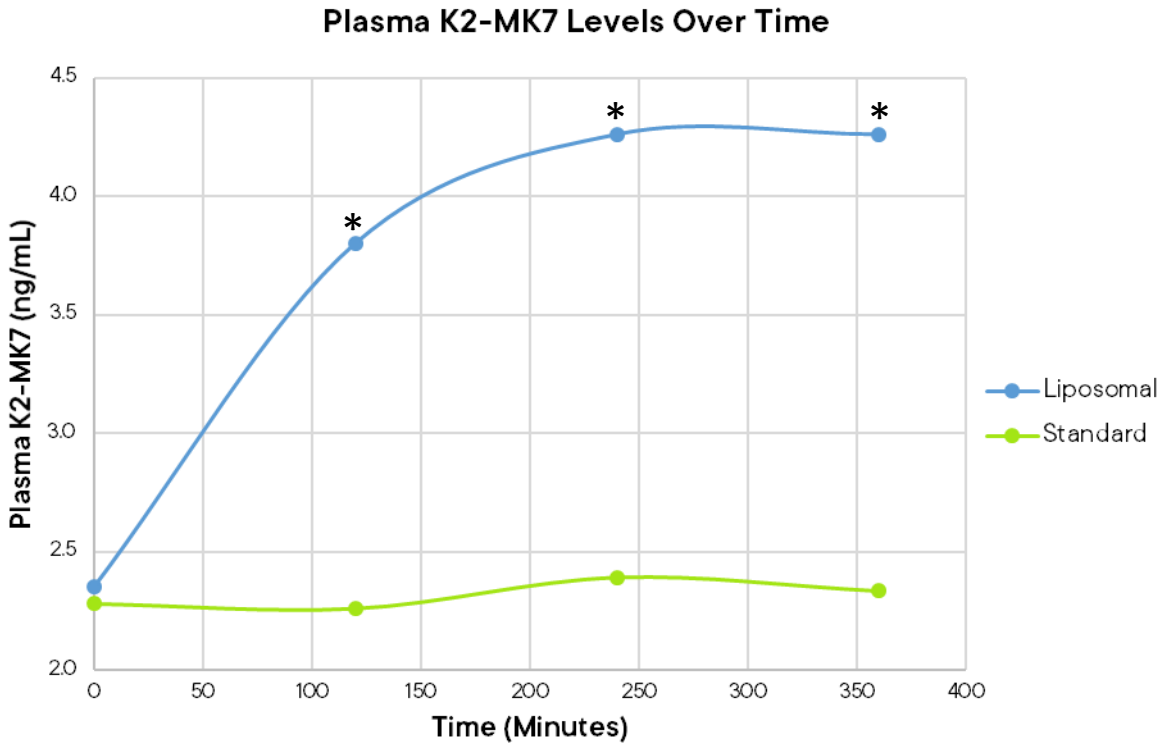

Figure 2. Plasma K2-MK7 levels over time after administration of 200mcg Vitamin K2-MK7 supplement in liposomal or standard form
* Indicates a significant difference between groups at the given time point (p<0.05)

4. Discussion

4.1. Vitamin D3

Pharmacokinetic data show that the liposomal vitamin D3 product was almost two times as bioavailable as the standard product. Between group analyses showed that the liposomal supplement had a high bioavailability and raised serum 25(OH)D levels higher than the standard product 120, 240, and 360 minutes after supplementation.

The levels of 25(OH)D from the liposomal group significantly increased after 120 minutes and remained elevated with no significant change in either direction for the duration of the 360-minute sampling period suggesting that the liposomal product was able to maintain elevated serum levels during this time.

The standard product saw a significantly increase in serum levels from baseline to 240 minutes, but levels than decreased at the following time point back to their original levels. The standard product was unable to increase serum levels to that of the liposomal product, nor was it able to maintain elevated levels for as long as a period of time.

It is possible that the true C_{max} of the vitamin D supplement was not fully reached within the 360 minutes of the study, as the value continued to rise at the 360-minute mark. In this case, the true OBV for liposomal vitamin D3 may be higher than reported. Future studies should evaluate serum 25(OH)D values for a longer time after administration of the single-dose.

When compared to the broader literature, the serum 25(OH)D levels reached from a single-dose of the 1,000IU liposomal product can be considered high. In a study conducted by Wagner et al. of a gummy versus tablet vitamin D3 supplement at a dose of 20,000 IUs, the gummy vitamin performed best with a C_{max} of 47.30 ng/mL after 9.70 hours.[1] A study conducted by Nandgaye et al. compared the bioavailability of an oral solution of vitamin D using nanotechnology with a tablet and capsule product of vitamin D3 at a dose of 60,000 IUs. The oral solution supplement performed best and serum 25(OH)D levels reached a C_{max} of 40.02 ng/mL at a T_{max} of 15.50 hours.[2] A study in 2010 by Coelho et al. examined the bioavailability of a 66,000 IU dose of a capsule in lactose excipient versus an oily drops of the same dose. The capsule performed best with a C_{max} of 30.16 ng/mL at a T_{max} of 14.00.[3]

These studies used dosages 20-66x higher than our liposomal product and were still unable to match the serum 25(OH)D levels reached by our liposomal product. This data shows the high bioavailability of our liposomal product when compared to competitors and suggests that liposomes can increase serum levels at lower dosages with higher efficiency and speed *(Table 9)*.

Table 9. Comparison of Vitamin D3 Bioavailability in Different Application Forms

Study Author	Application Form	Dose (IUs)	C_{max} (ng/mL)	T_{max} (hours)
PlantaCorp GmbH, 2020	**Liposomal**	**1,000**	**52.90**	**6.00**
	Pinnacle Nutrition Tablet	1,000	25.60	4.00
Wagner et al., 2019[1]	VitaFusion Gummy	20,000	47.30	9.70
	Nature Made Tablet	20,000	23.40	9.50
Nandgaye et al., 2018[2]	Hi-D Oral Solution	60,000	40.02	15.50
	Must Tablets	60,000	35.41	19.50
	Uprise Soft Gelatin Capsules	60,000	34.79	19.00
Coelho et al., 2010[3]	Capsule in Lactose Excipient	66,000	30.16	15.11
	Oily Drops	66,000	28.51	14.00

IU = International Units

C_{max} = Maximum blood concentration of vitamin D that was reached after administration of a single dose.

T_{max} = The time the maximum concentration was reached.

4.2. Vitamin K2-MK7

Pharmacokinetic data show that the liposomal vitamin K2-MK7 product was 1.63 times as bioavailable as the standard product. Between group analyses showed that the liposomal

supplement had a high bioavailability and raised plasma K2-MK7 levels higher than the standard product at 120, 240, and 360 minutes after supplementation.

The liposomal product raised plasma levels significantly from baseline and maintained these levels with no change in either direction for the duration of the 360-minute sampling period. The standard product was unable to elicit any significant change in plasma levels from baseline. The ability of the liposomal product to produce significant changes in plasma K2-MK7 levels is notable in this study.

Due to complications in the laboratory analysis of the plasma K2-MK7, the measurements recorded may not be the absolute values. It is estimated that roughly 50% of K2-MK7 in the blood was not detected by the analysis. However, this was uniform across all samples, and thus the comparison of values between groups is still valid.

4.3. Conclusions

The liposomal vitamin D3K2 supplement significantly raised both serum 25(OH)D and plasma K2-MK7 levels from baseline after 120, 240, and 360 minutes when compared to a standard vitamin D3K2 supplement and were able to maintain these elevated levels through the 360 minute sampling period. The use of a combined supplement may have affected the absorption of the individual nutrients positively or negatively in each study group and should be considered in the presentation of results.

Overall, it can be concluded that the liposomal vitamin D3K2 product is significantly more bioavailable than a standard product at the same dose and is the more efficient option for supplementation.

References

1. Wagner CL, Shary JR, Nietert PJ, Wahlquist AE, Ebeling MD, Hollis BW. Bioequivalence studies of vitamin D gummies and tablets in healthy adults: Results of a cross-over study. *Nutrients.* 2019;11(5). doi:10.3390/nu11051023

2. Nandgaye KM, Samant RS, Kadam SB, Palkar PJ. Relative oral bioavailability of three formulations of vitamin D3: An open-label, three-treatment study. *Int J Basic Clin Pharmacol.* 2018;8(1):138. doi:10.18203/2319-2003.ijbcp20185172

3. Coelho IMG, Andrade LD de, Saldanha L, Diniz ET, Griz L, Bandeira F. Bioavailability of vitamin D3 in non-oily capsules: the role of formulated compounds and implications for intermittent replacement. *Arq Bras Endocrinol Metabol.* 2010;54(2):239-243. doi:10.1590/s0004-27302010000200022

Acknowledgements

Study conducted in collaboration with:
Surya Research Clinics
C-6, Sujan Singh Park
Cornwallis Road
New Delhi, Delhi 110003
J62H+CF New Delhi, Delhi, India