BIOAVAILABILITY OF BERBERINE AND DIHYDROBERBERINE

A statistical report submitted to:

NNB NUTRITION

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Overview of Report

Purpose:

To determine the bioavailability of a 500 mg dose of berberine and 100 and 200 mg doses of dihydroberberine.

Specific Aims

I. To identify and compare the appearance of berberine in collected blood samples after ingestion of a 500mg dose of berberine, 100mg dose of dihydroberberine, and a 200mg dose of dihydroberberine.

Independent Variable

- Supplementation Groups
  - Placebo
  - 500 mg Berberine
  - 100 mg Dihydroberberine
  - 200 mg Dihydroberberine

Dependent Variables

Primary Endpoint

- Plasma concentrations of berberine

Secondary Endpoints

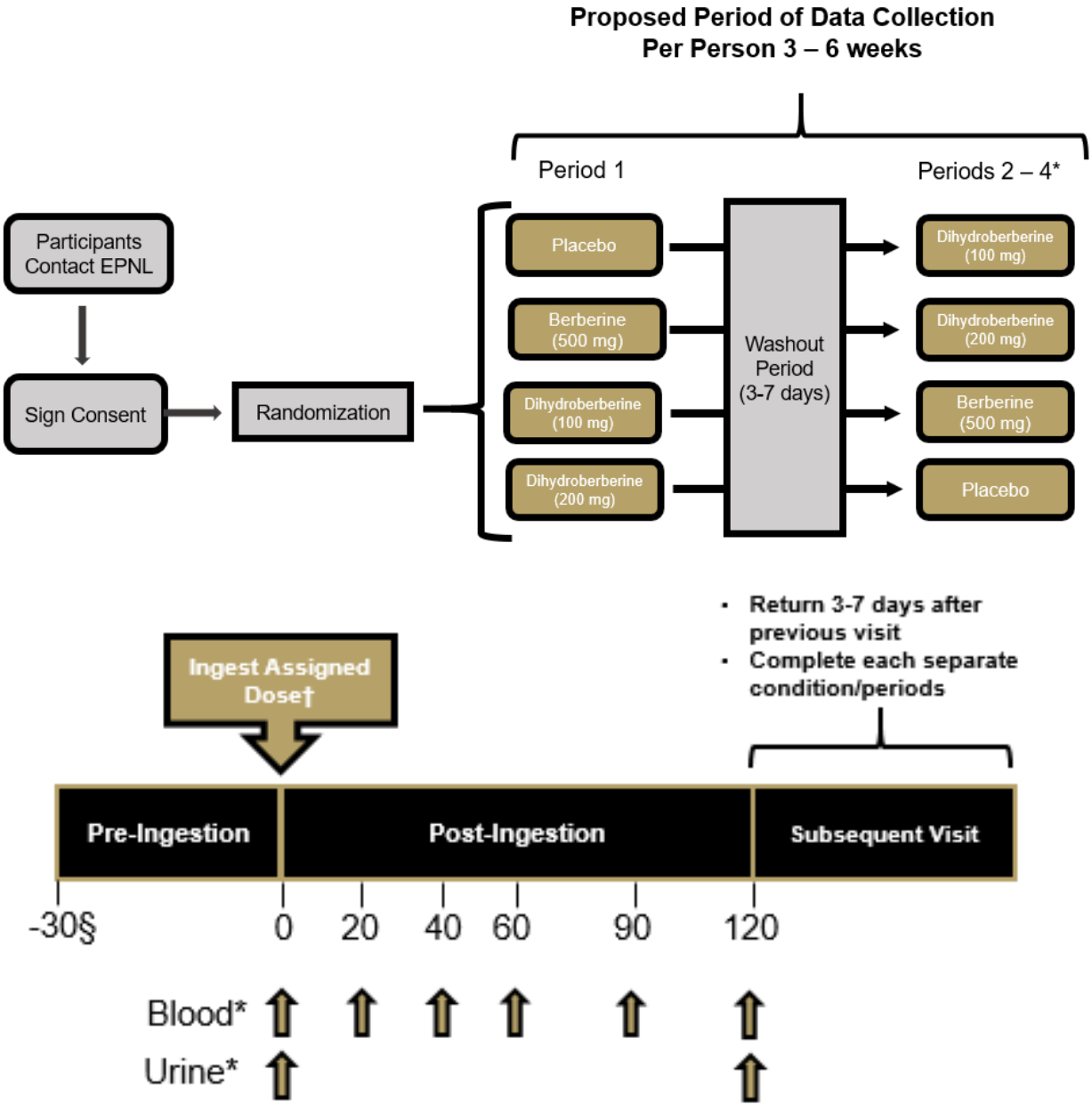
- Safety Assessment (heart, rate, blood pressure, complete blood count, and comprehensive metabolic panel, and urinalysis)
- Incidence and associated severity of reported adverse events

Blinding

All capsules were blinded by a PhD trained faculty colleague on 9/17/20. The envelope was signed and was retained in the principal investigator's office and was unblended on 2/4/21.

A = Placebo (PLA)
B = 100 mg Dihydroberberine (D100)
C = 200 mg Dihydroberberine (D200)
D = 500 mg Berberine (B500)

Overview of Study Design



- In a randomized, double-blind, crossover fashion, study participants followed one of three supplementation conditions after an overnight fast. Venous blood samples were collected 0, 20, 40, 60, 90, and 120 minutes after final dose and test meal.
- Adverse events were reported via questionnaire and to study team members throughout the study trial.
- Participants ingested four total doses of their assigned supplement. Starting with the morning before their scheduled visit, participants ingested one dose with breakfast, one dose with lunch, and one dose with dinner. Participants were instructed to ingest all doses with eight fluid ounces of cold water. Participants followed an overnight (8 – 10 hours) fast and consumed their final dose upon arriving in the research laboratory with

their standardized test meal. Water consumption was encouraged during the fasting period. In summary, all participants ingested a total of four doses for each of the assigned supplementation conditions prior to consuming their test meal.

Statistical Approach

- Descriptive statistics were calculated using standard statistical methods. All data are presented as means ± SD.
- Outlier analysis was completed using the calculation of studentized residuals for all raw values of berberine. Additionally, the change from baseline scores (deltas) were also assessed for outliers. A threshold of ±3 was used for determination of a statistical outlier.
    - *No values were deemed to be statistical outliers.*
- Area under the curve for each amino acid was calculated using the trapezoidal method (Equation 1), where C_0 is the unknown concentration at the first time point of interest, C_1 is the unknown concentration at the second time point of interest, and $Time_{1\text{-}0}$ is the total time interval between the two time points when the unknown was measured. These individual AUC values for each time interval were then summed to determine the total AUC for each amino acid (Equation 2).

Equation 1: $$\mathrm{AUC}_1 = \frac{\mathrm{C}_0+\mathrm{C}_1}{2} \times \mathrm{Time}_{1-0}$$

Equation 2: $$\mathrm{AUC}_{\mathrm{Total}} = \mathrm{AUC}_1 + \mathrm{AUC}_2 + \mathrm{AUC}_3 + \cdots + \mathrm{AUC}_{\mathrm{n}}$$

- The significance threshold for all hypothesis testing was set at an alpha level of $p < 0.05$
- All AUC were computed using Microsoft Excel and all means, standard deviations, normality and independent t-tests were computed using SPSS v26 (New York, USA)
- Mixed factorial ANOVA (group x time) were completed for assessment of all changes in berberine and health markers.

Supplement Compliance

Participants were asked to abstain from food and drink overnight in addition to abstaining from alcohol and nicotine for approximately 12 hours. Finally, participants were asked to abstain from exercise for 24 hours prior to each visit. All supplemental conditions were consumed in a double-blind fashion with a research team member observing all ingestions. Subsequently, 100% compliance to the supplementation protocol is reported.

Executive Summary

Using a randomized, double-blind, placebo-controlled, crossover approach, we report that:

- Four doses of 200 mg of dihydroberberine leads to significantly higher area under the curve values as compared to those values observed for placebo and 500mg of berberine.
- Four doses of 100 mg of dihydroberberine leads to significantly higher area under the curve values as compared to those values observed for placebo and 500mg of berberine.
- All supplementation is well tolerated with no clinically significant changes in blood-based markers of health and reported adverse events.

Figure 1. Berberine Raw Data (All Values Retained)

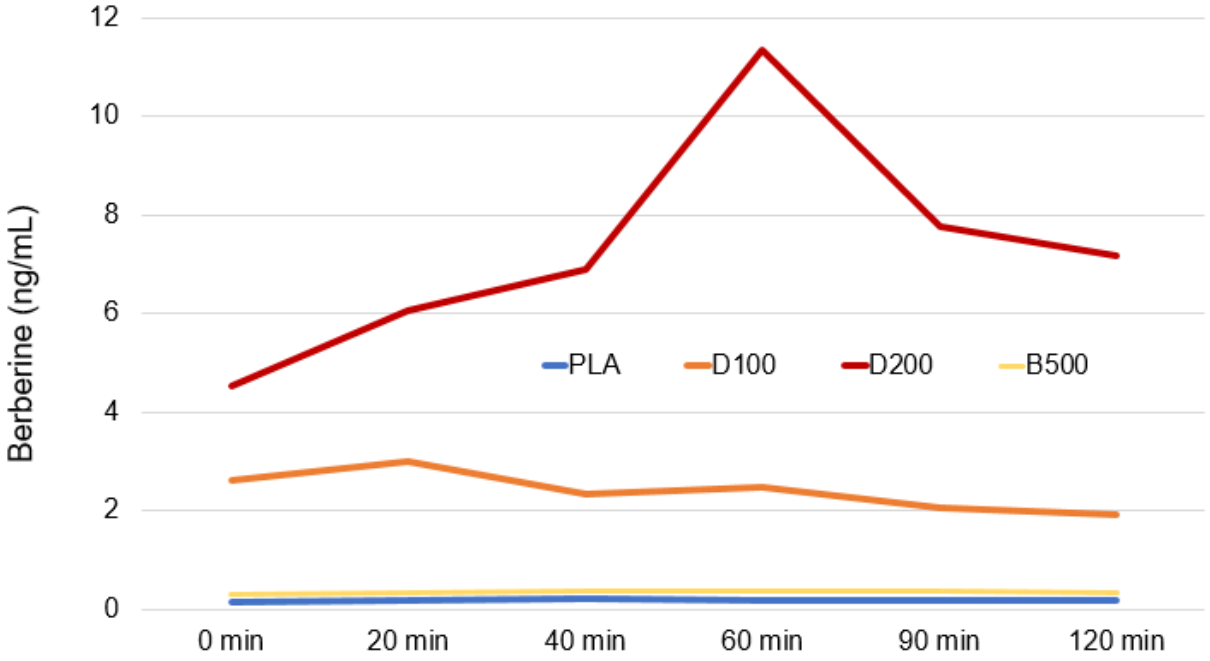
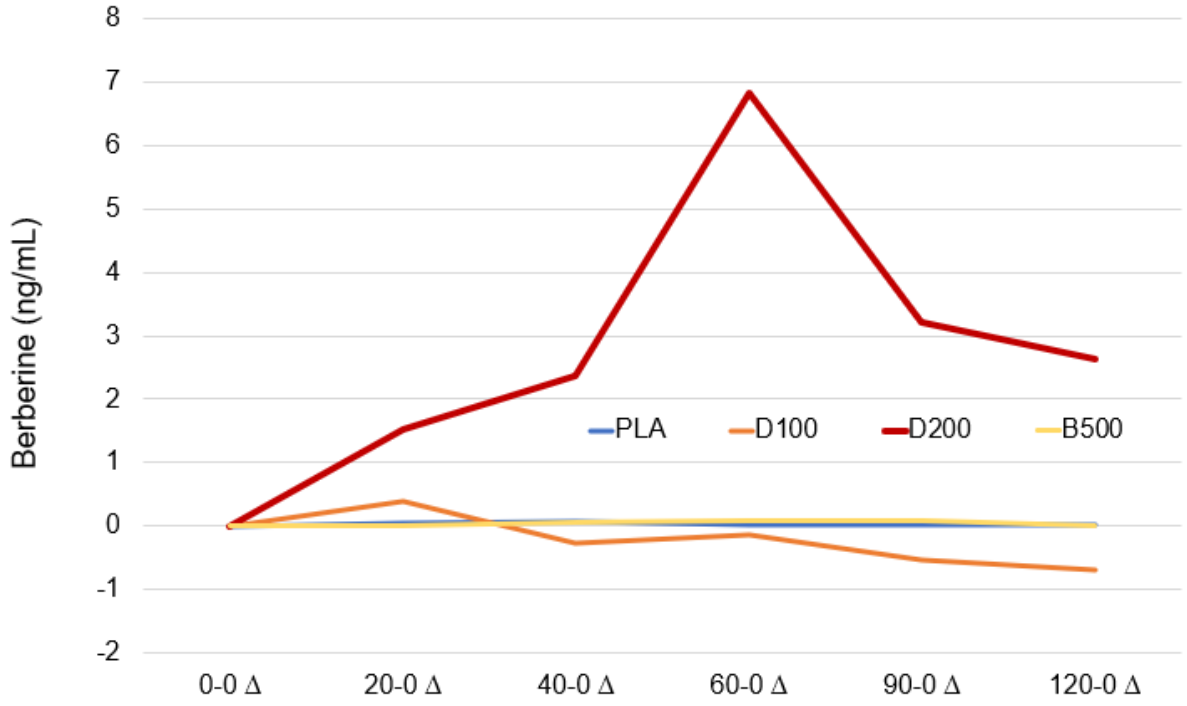
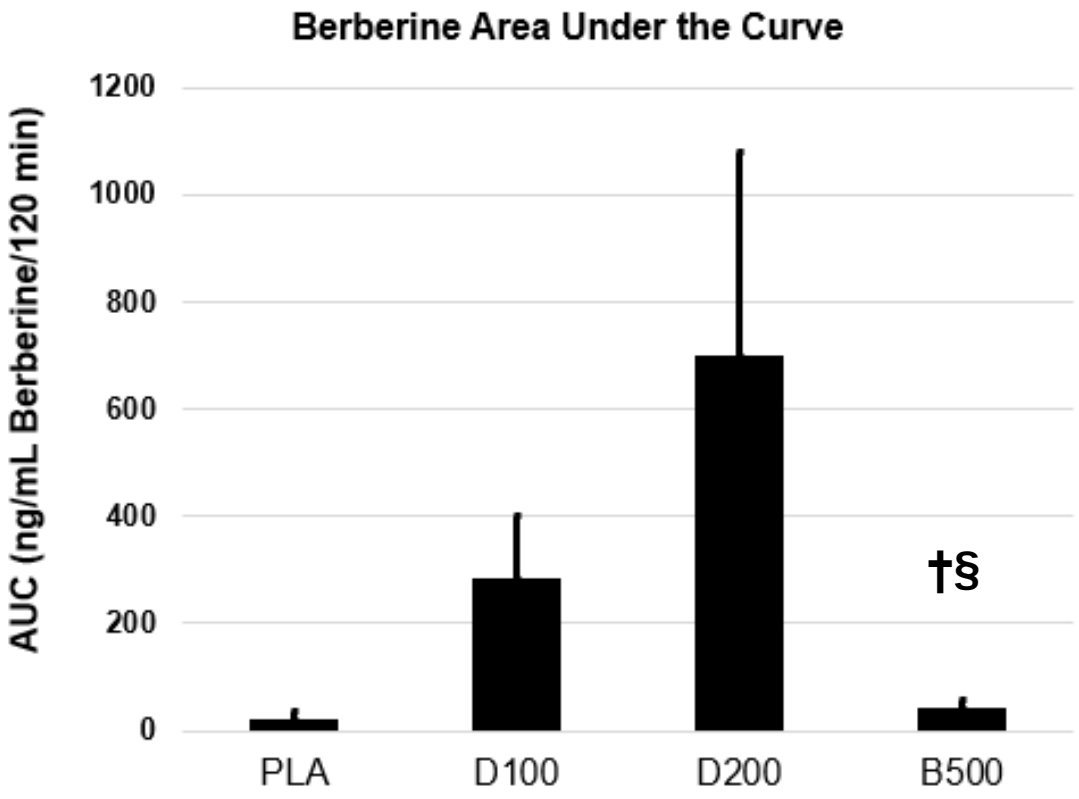


Figure 2. Berberine – Changes from Baseline (All Values Retained)



% Change Between Calculated Area Under the Curve Values

Condition		Condition	% Difference
PLA	vs.	D100	PLA AUC was 1307% lower than the D100 AUC
		D200	PLA AUC was 3359% lower than the D200 AUC
		B500	PLA AUC was 109% lower than B500 AUC
D100	vs.	PLA	D100 AUC was 1307% higher than the PLA AUC
		D200	D100 AUC was 146% lower than the D200 AUC
		B500	D100 AUC was 572% higher than the B500 AUC
D200	vs.	PLA	D200 AUC was 3359% higher than the PLA AUC
		D100	D200 AUC was 146% higher than the D100 AUC
		B500	D200 AUC was 1552% higher than the B500 AUC
B500	Vs.	PLA	B500 AUC was 109% higher than the PLA AUC
		D100	B500 AUC was 572% lower than the D100 AUC
		D200	B500 AUC was 1552% lower than the D200 AUC



† = Different than D100 ($p < 0.05$).
§ = Different than D200 ($p < 0.05$).

Figure 3.1. Berberine Area Under the Curves
Each dot represents one of the study participants who completed the study protocol. The black horizontal bar represents the average AUC value for each condition. NOTE: The scale for the two figures on the left (0 – 100) is must different than the scale for the two figures on the right (0 – 2000)