

Riboflavin Concentrations in Lactating Women over the First Eight Months Postpartum

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Background

- Riboflavin (vitamin B2) is an essential vitamin that plays a role in energy metabolism, cellular function, and infant growth.¹
- Riboflavin is excreted into breastmilk in lactating women and is an essential infant nutrient.²
- Riboflavin degrades when exposed to visible or UV light.^{3,4}
- A previous study reported plasma riboflavin mean concentrations to be 12.7 – 12.9 nM in women 6 months postpartum.⁵

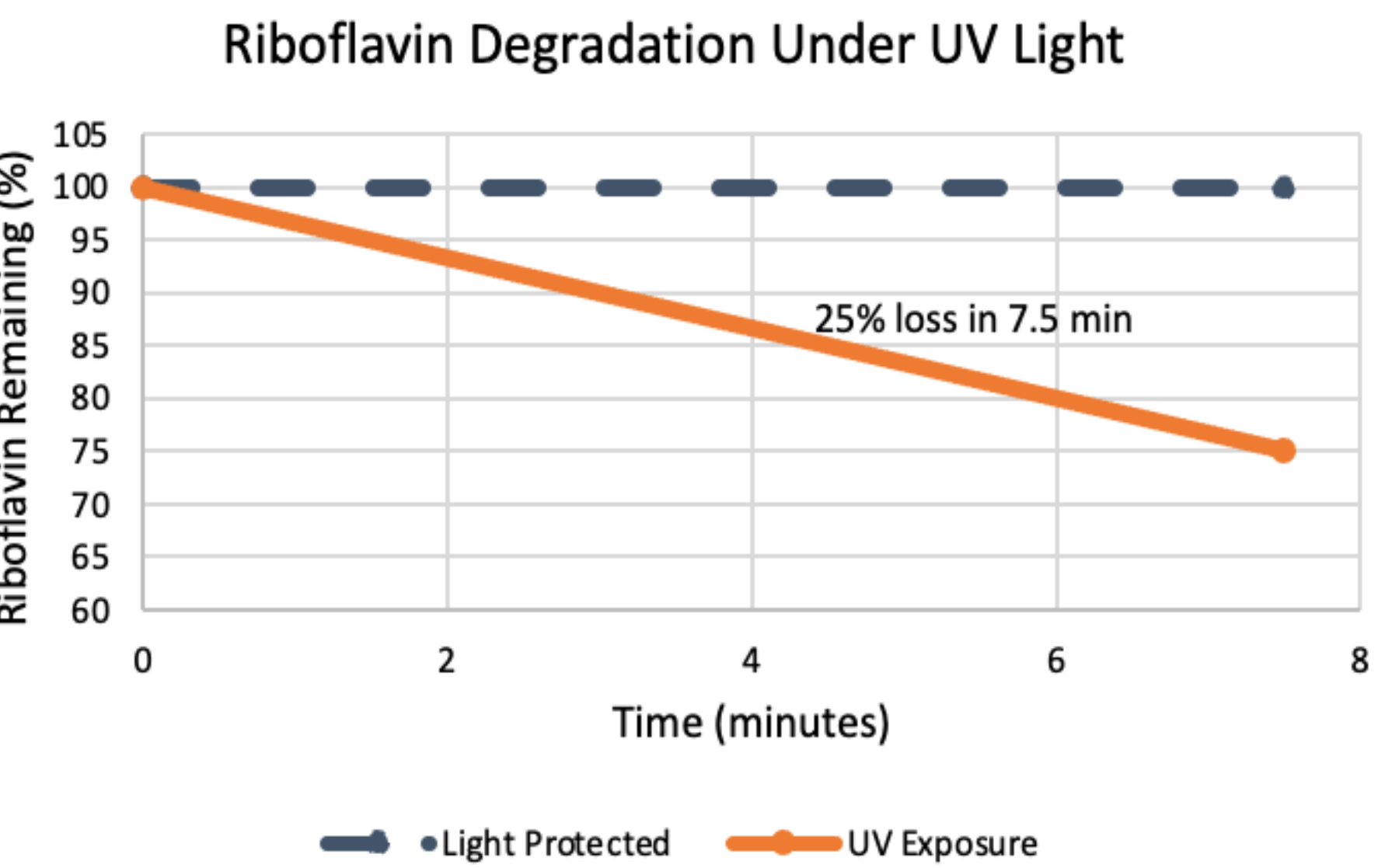


Figure 1. Riboflavin degradation by UV light over time. There is approximately 25% loss in riboflavin concentrations in 7.5 minutes of UV exposure.⁴

Objectives

- Comparing riboflavin concentrations in lactating women 3-5 weeks, 3-4 months, and 6-8 months postpartum.
- Evaluating the relationship between riboflavin supplementation dose and riboflavin area under the concentration-time curve (AUC).

Methods

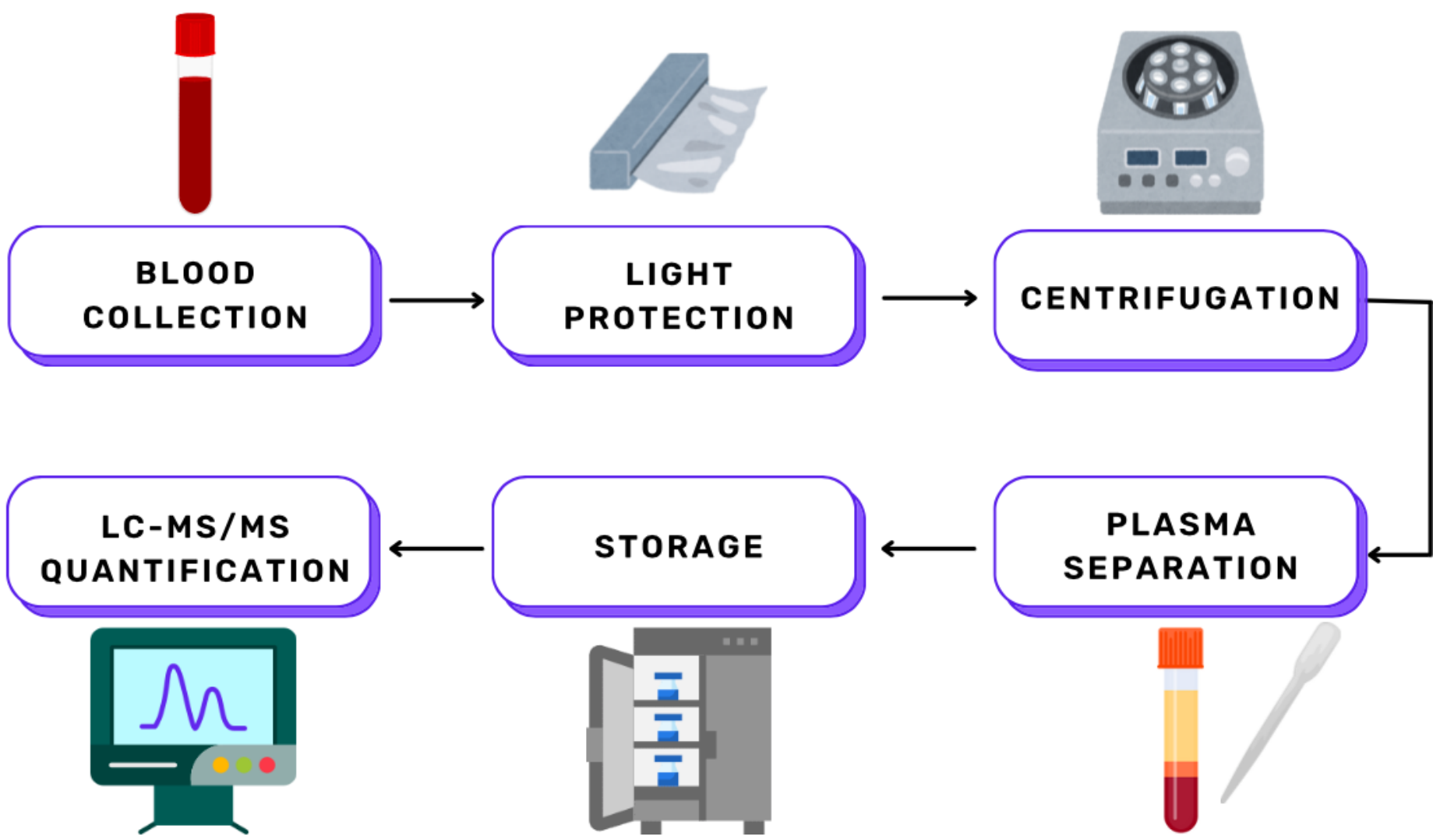


Figure 2. General study design. Figure created by the author using Canva.

Methods, contd.

- Participants: 8 lactating women completed 3 study days (3-5 weeks, 3-4 months, 6-8 months postpartum).
- Sample collection: 10 blood samples collected in heparinized tubes. Samples wrapped in foil, placed on ice, and processed under UV-minimized conditions.
- Processing: Blood samples were centrifuged at 4 °C for 10 min at 3000 g. Plasma separated and stored at -80 °C in amber cryovials
- Analysis: Plasma riboflavin concentrations were quantified using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. Riboflavin AUC was estimated using trapezoidal rule.
- Statistics: Two-tailed paired Student's t-test was used to compare AUCs across windows. Pearson Correlation analysis between riboflavin and plasma riboflavin AUC was completed. $p < 0.05$ was considered statistically significant.

Results

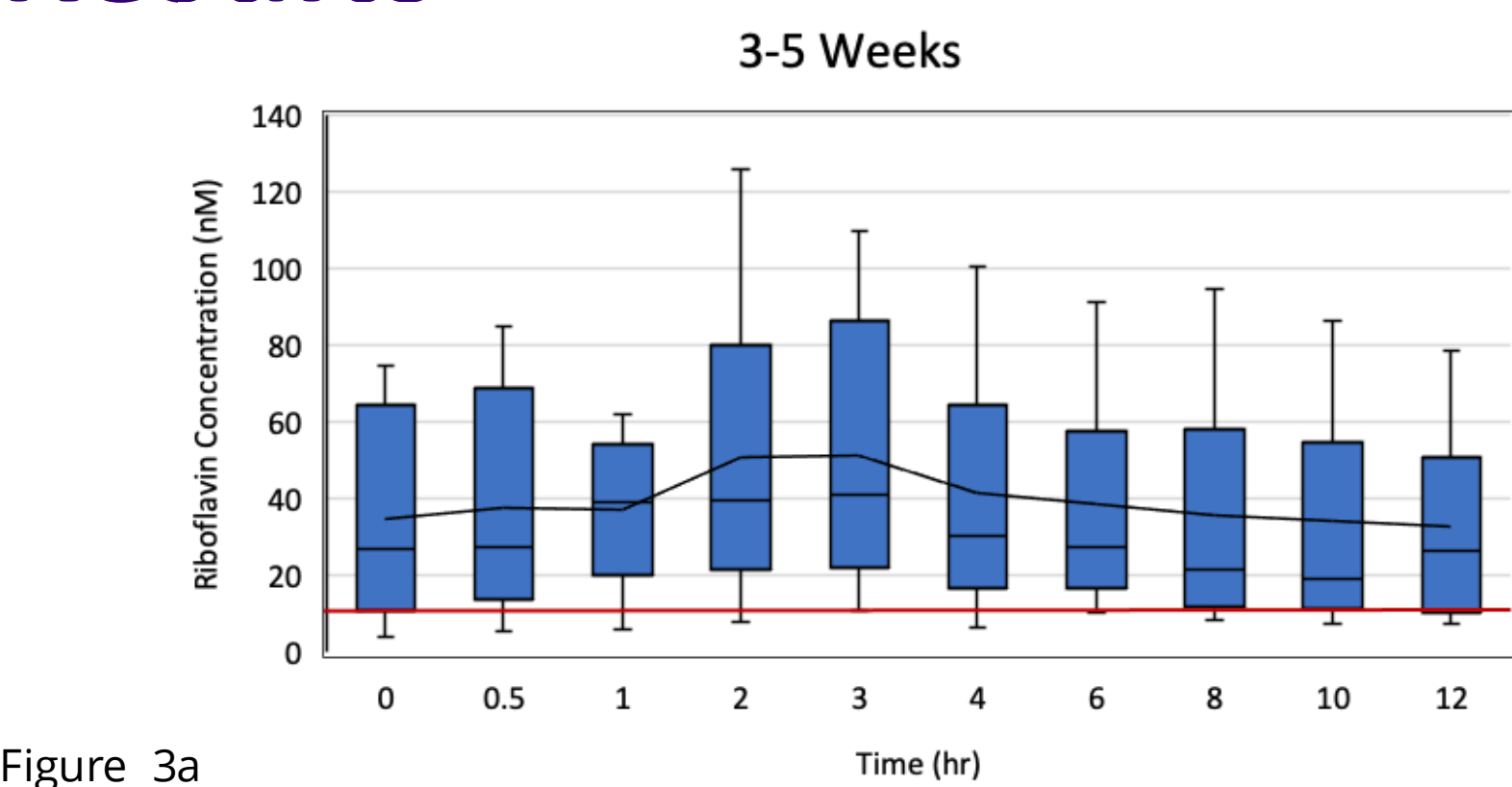


Figure 3a

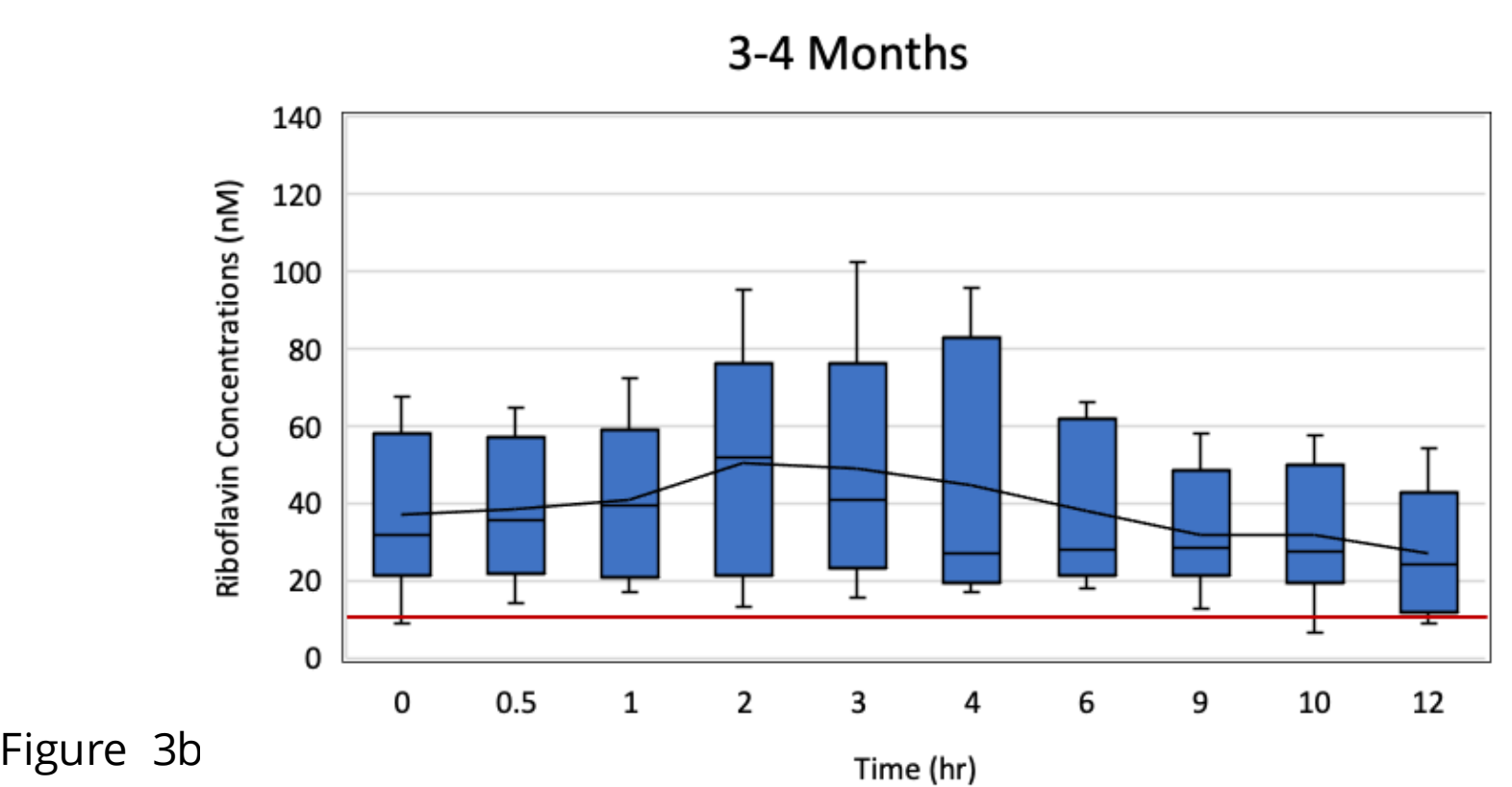


Figure 3b

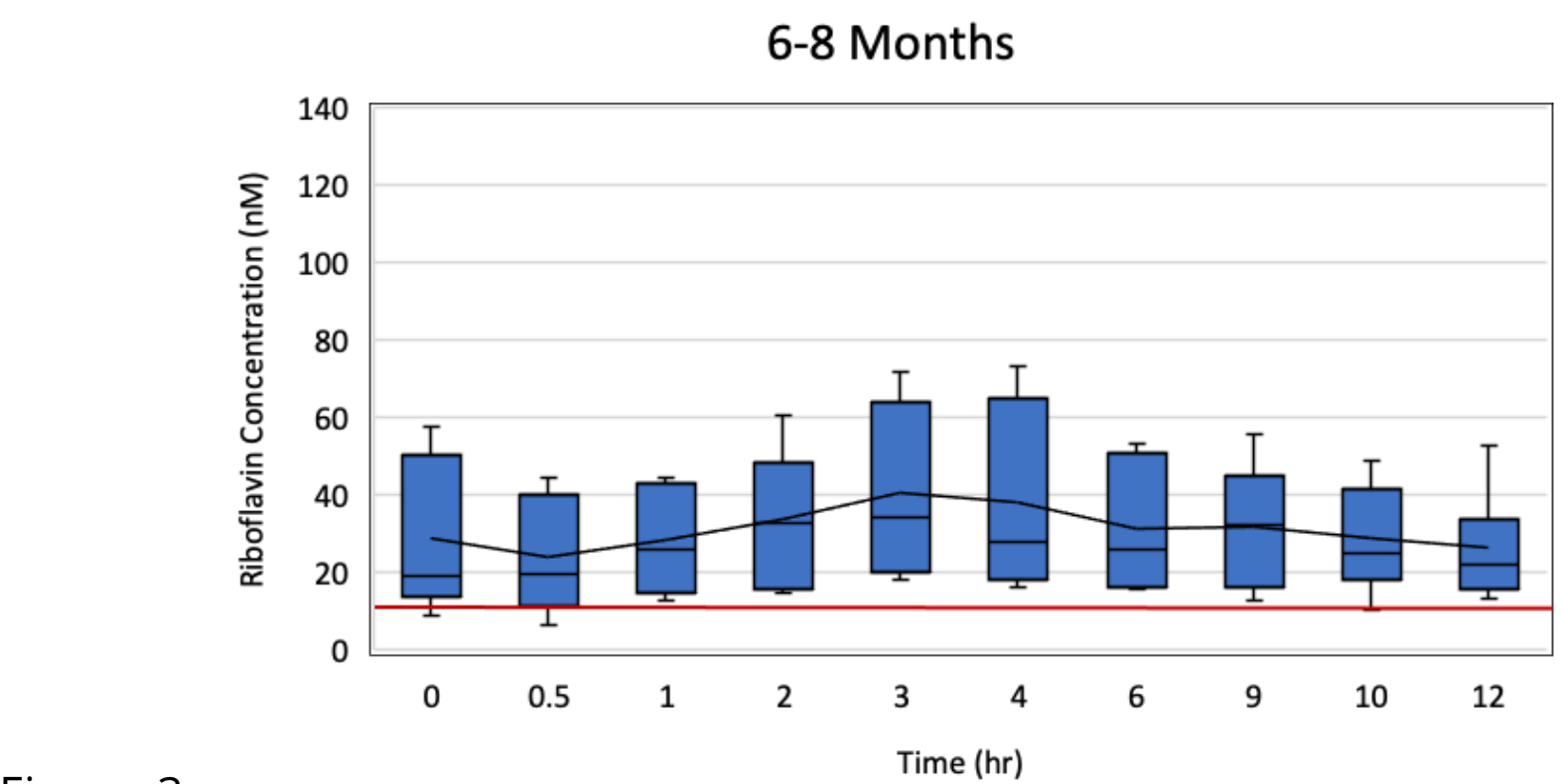


Figure 3c

Figure 3. Box and whisker plots showing plasma riboflavin concentrations at 3-5 weeks (figure 3a), 3-4 months (figure 3b), and 6-8 months (figure 3c). The trendline represents the mean value, the horizontal line in each rectangle represents the median value, and the edges of the rectangle indicate the 25th and 75th percentiles. The upper and lower whiskers represent the maximum and minimum values within 1.5× the interquartile range. The red horizontal line indicates the mean plasma concentration measured 6 months postpartum as previously reported by Godfrey et al. (12.8 nM).⁵

Results, contd.

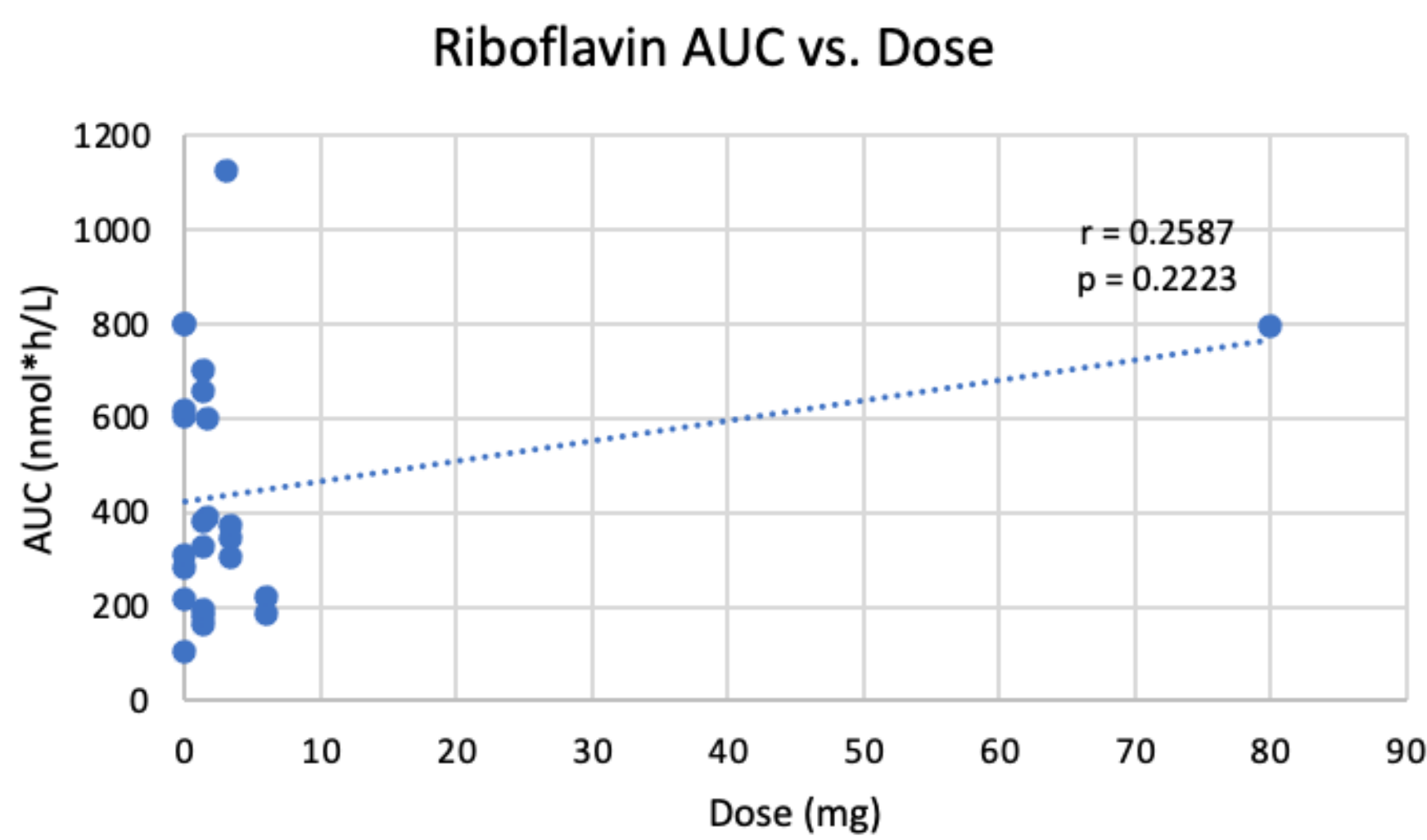


Figure 4. Relationship between riboflavin area under the curve (AUC) and reported supplemental dose across all study days. Points represent individual participants, with Pearson correlation analysis, performed in Excel (p-value).

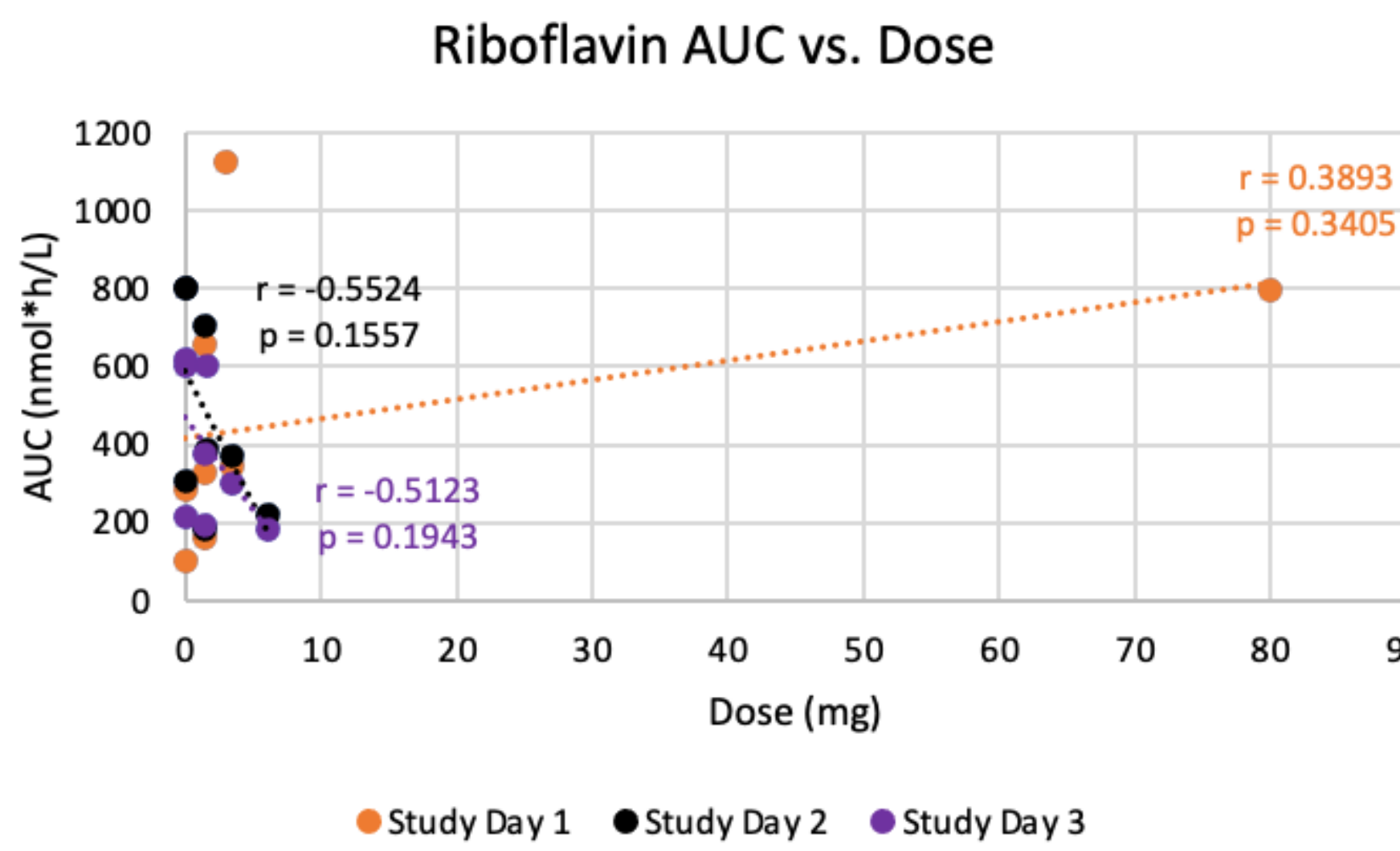


Figure 5. Riboflavin AUC versus riboflavin supplement dose by study day (3-5 weeks, 3-4 months, 6-8 months postpartum). Points represent individual participants, and separate regression lines are shown for each day. Pearson correlation analysis, performed in Excel.

- There is high variability in riboflavin concentrations across the first 8 months postpartum.
- Mean $\pm$ SD riboflavin AUCs were 474.9 ± 352.3 nmol*h/L at 3-5 weeks; 471.9 ± 256.6 nmol*h/L at 3-4 months; and 387.4 ± 193.0 nmol*h/L at 6-8 months.
- There was no significant difference in riboflavin AUC between 3-5 weeks and 6-8 months postpartum. $p = 0.25$.
- Riboflavin AUC at 3-4 months postpartum was significantly higher than 6-8 months postpartum. $p = 0.02$.
- Overall, there was not a significant correlation between riboflavin supplementation dose and riboflavin AUC. $p = 0.2$ (data not shown).

Discussion

- Plasma riboflavin concentrations in our study were higher than values reported by Godfrey et al. ($n = 1,729$ women 6 months postpartum). The study was conducted in the UK, Singapore, and New Zealand. This study included no riboflavin supplementation (mean riboflavin plasma concentration 12.9 nM) and with 1.8 mg/day supplementation (mean riboflavin plasma concentration 12.7 nM).⁵ Light protection during plasma handling was not reported by Godfrey et al.⁵
- The differences in riboflavin concentrations reported by Godfrey et al. and our study are not surprising given riboflavin can degrade by ~25% within minutes after UV/visible light exposure.⁴ Light-protected handling in our study likely reduced degradation resulting in higher concentrations.
- Consistent with previously reported data⁵, we found no significant correlation between riboflavin supplementary dose and overall riboflavin exposure.

Conclusion

- The methodological differences in sample handling processes likely is responsible for falsely low concentrations being reported by other investigators.
- Riboflavin concentrations are significantly higher 3-4 months postpartum than 6-8 months postpartum
- Consistent with previous reports, riboflavin supplementation did not correlate with riboflavin AUC.

Future Directions

- Explore the mechanism by which riboflavin concentrations change postpartum.
- Evaluate long-term stability of riboflavin in stored plasma under different protection and storage conditions.

Acknowledgements

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References

- Powers HJ. 2003. Riboflavin (vitamin B-2) and health. *The American Journal of Clinical Nutrition*. 77(6):1352-1360.
- Dror DK, Allen LH. 2018. Overview of Nutrients in Human Milksecondary analysis of the NiPPeR double-blind randomized controlled trial. *PLoS Med* 20(12): e1004260. *Advances in Nutrition*. 9(suppl_1):2785-2945.
- Sheraz et al. 2014. Photo, thermal and chemical degradation of riboflavin. *Beilstein Journal of Organic Chemistry*. 10(1):1999-2021.
- Ahmad I, Fasihullah Q, Vaid FHM. 2006. Effect of light intensity and wavelengths on photodegradation reactions of riboflavin in aqueous solution. *Journal of photochemistry and photobiology B, Biology*. 82(1):21–27.
- Godfrey KM, Titcombe P, El-Heis S, Albert BB, Tham EH, Barton SJ, et al. (2023) Maternal B-vitamin and vitamin D status before, during, and after pregnancy and the influence of supplementation preconception and during pregnancy: Prespecified secondary analysis of the NiPPeR double-blind randomized controlled trial. *PLoS Med* 20(12): e1004260.