



# Determination of Microsomal And Cytosolic Scaling Factors in the Female Yucatan Minipig



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## Introduction:

- *In Vitro In Vivo* Extrapolation (IVIVE) is a rapidly emerging gold standard for conversion of pharmacokinetic (PK) data from benchtop to clinic<sup>1</sup>. IVIVE is best deployed in dosage regimens for first-in-human clinical trials, or for re-purposing drugs in special populations<sup>2</sup>. This method is advantageous as it eliminates the need for live animal testing.
- Essential to IVIVE are the scaling factors, Microsomal Protein Per Gram Liver (MPPGL) and Cytosolic Protein Per Gram Liver (CPPGL)<sup>2</sup>. MPPGL and CPPGL scalars are best defined for humans, rats, and dogs, leaving other animal species poorly defined. One such prominent animal is the minipig, of which is an incredibly effective model of cardiovascular, digestive, urogenital, and epidermal pharmacology<sup>3</sup>. Scalar determination is also inconsistent with methodological consideration of freeze thaw cycling on protein content.

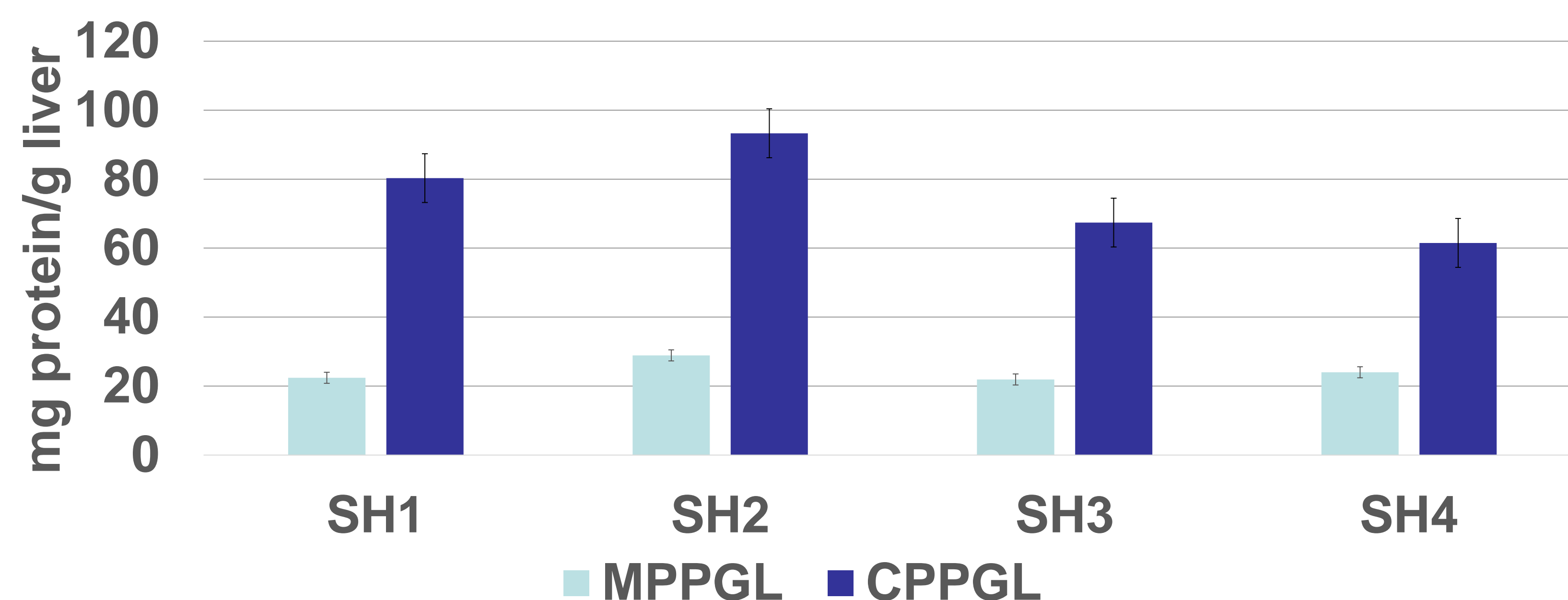
## Objectives:

- Given the lack of scalar data on the minipig, and the inconsistent consideration of freeze thaw effects, we set out to:
  1. Define MPPGL and CPPGL scalars for the female Yucatan minipig.
  2. Assess the effect of freeze thaw cycling on scalar protein content.

## Methods:

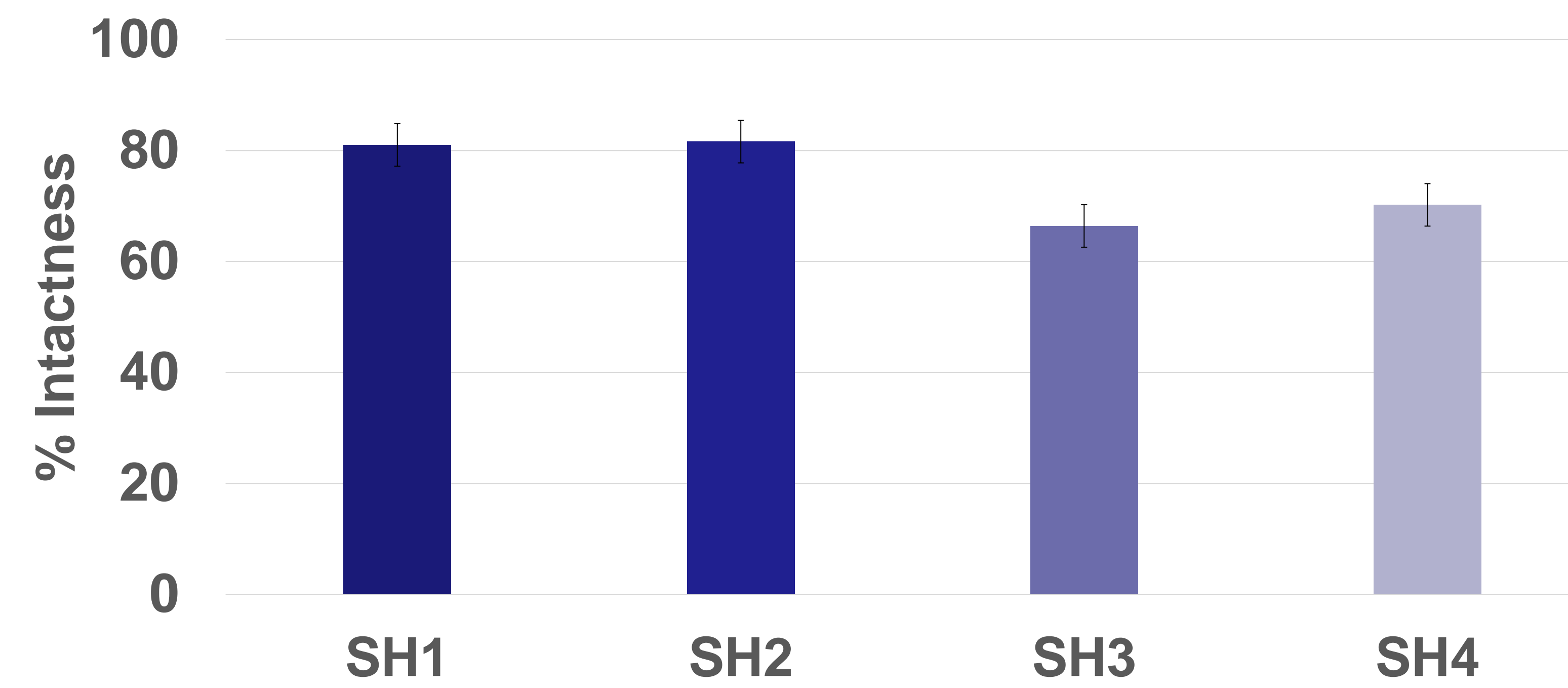
- All tissue samples were harvested as waste products from prior animal studies. Liver tissue was then thawed on ice, homogenized in sucrose HEPES (SH) buffer, and centrifuged (10,000 x g, 20 mins, 4°C) to yield S9 fraction. S9 supernatant was then ultracentrifuged (100,000 x g, 75 mins, 4°C) to yield microsomal and cytosolic fractions. Protein content was estimated via Bicinchoninic Acid (BCA) assay of respective fractions.
- Microsomal integrity was also probed to estimate microsomal purity. This was accomplished by a mannose-6-phosphatase activity assay.
- Freeze thaw effects were explored for n=3 cycles by BCA and Bradford assays respectively.

## Determination of MPPGL and CPPGL:



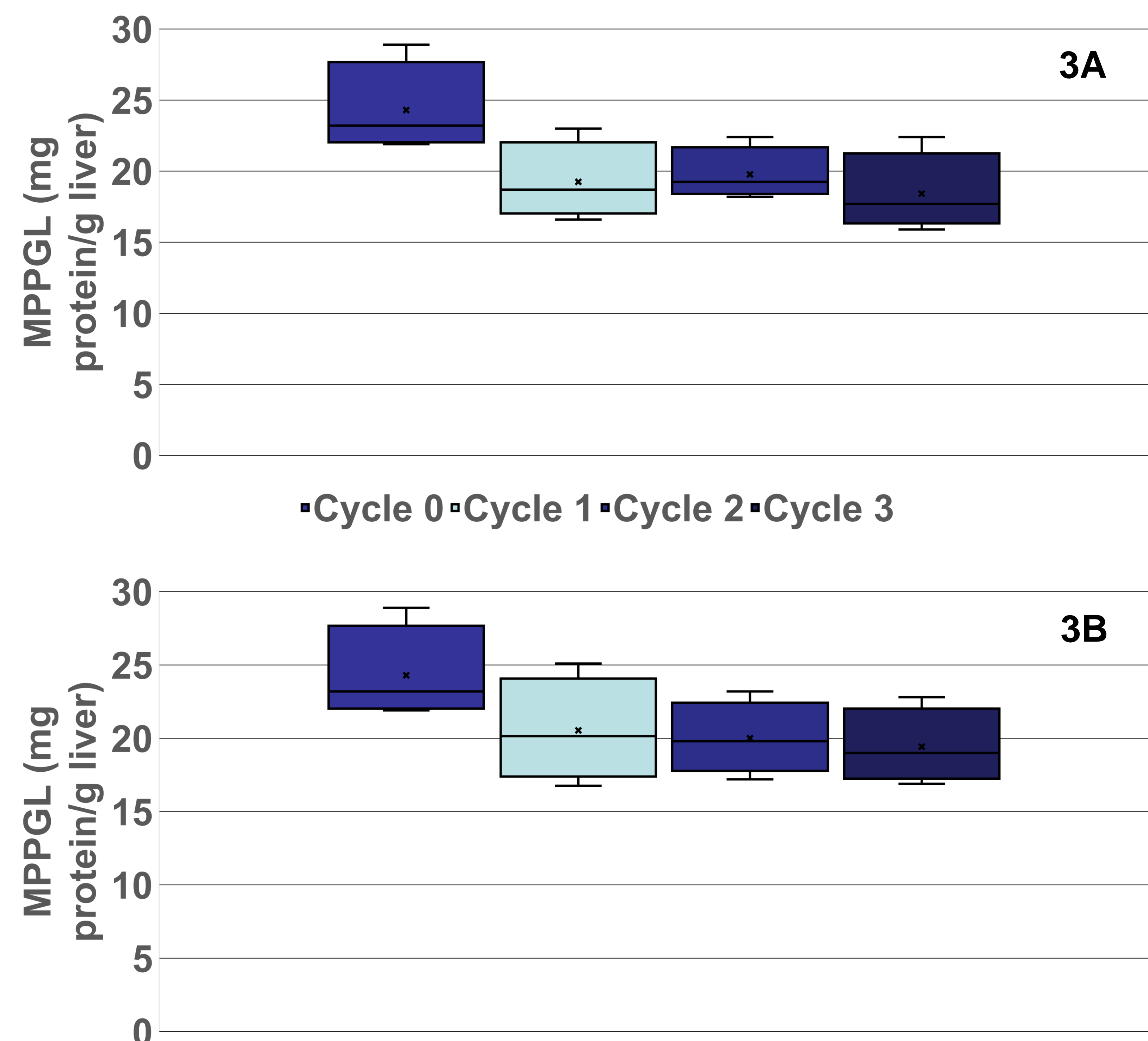
**Figure 1. MPPGL and CPPGL values.** Scalar values were defined for n=4 liver samples, where SH refers to the buffer used in preparation. Mean MPPGL and CPPGL values were 24.3 ± 3.2mg protein/g liver, and 75.6 ± 14.2mg protein/g liver respectively.

## Assessment of Microsomal Intactness:



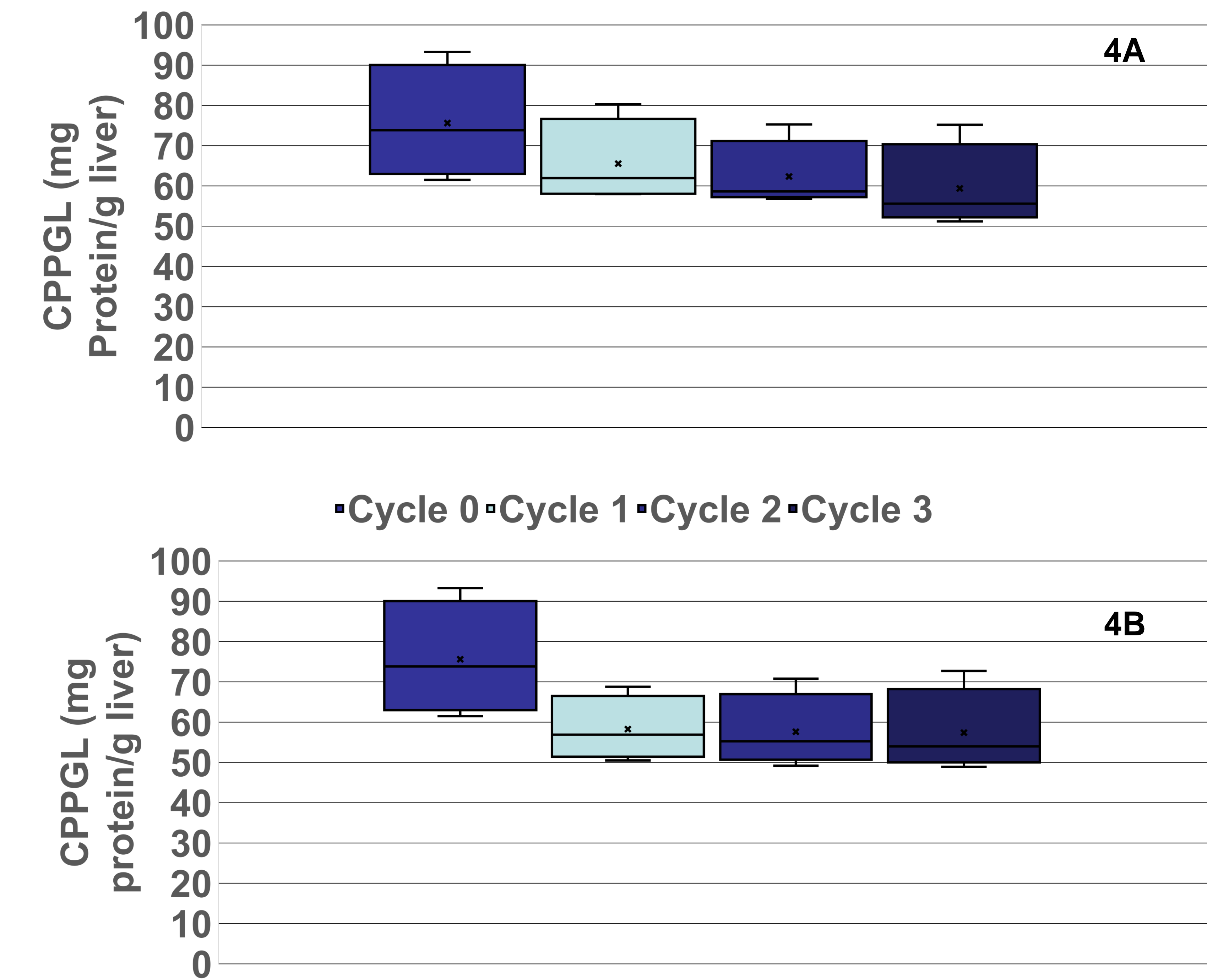
**Figure 2. Microsomal intactness.** Given the position of mannose-6-phosphatase within the microsomal membrane, activity was dependent on membrane integrity. Activity was measured in units of umol phosphate/min/mg protein. Mean microsomal intactness was derived at 74.8 ± 7.6%.

## Freeze Thaw Cycle Effect on MPPGL:



**Figure 3. Effect of freeze thaw cycling on MPPGL values.** Figure 3A reflects freeze thaw effects via BCA assay, whereas Figure 3B reflects freeze thaw effects via Bradford assay. X denotes the mean MPPGL for each cycle.

## Freeze Thaw Cycle Effect on CPPGL:



**Figure 4. Effect of freeze thaw cycling on CPPGL values.** Figure 4A reflects freeze thaw effects via BCA assay, and Figure 4B via Bradford assay. X denotes the mean CPPGL for each cycle

## Discussion:

- Minipig MPPGL and CPPGL scalars have been derived for the first time. Having this data will vastly reduce minipigs used in testing by enabling IVIVE modeling.
- MPPGL values appear to be consistent with findings for *Sus. Scrofa domestica* as per findings by Elmorsi and Millecam<sup>4,5</sup>.
- Variation between scalar values may be resultant from prior animal health status and hypoxic ischemic time. Controlling for these may reduce variation in future studies.
- Freeze thaw effects are not significant for MPPGL, but are for CPPGL over n=1 cycles, with the effect leveling off over time. Subsequently, scalars should be derived prior to freezing of microsomes and cytosol.
- Future directions include exploration of scalars in other minipig strains, and the effect of fresh harvesting vs prior freezing on scalar value.

## References:

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