VALUES OF HUMAN HEPATOCELLULARITY PER GRAM OF LIVER AND ASSOCIATED VARIABILITY FOR USE IN THE PREDICTION OF HUMAN *IN VIVO* METABOLIC CLEARANCE

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INTRODUCTION

- Extrapolation of data from human hepatocytes to predict *in vivo* drug clearance (IVIVE) invariably assumes a fixed value of hepatocellularity per gram of human liver (HPGL) without accounting for inter-individual variability.
- The most commonly used value of 120 x 10^6 cells g^{-1} [1] (Figure 1) was not determined experimentally and, from personal communication with the original source, appears to have been only a guess.
- The aim of this study was to provide researchers with experimentally determined values of human HPGL together with a measure of inter-individual variability.

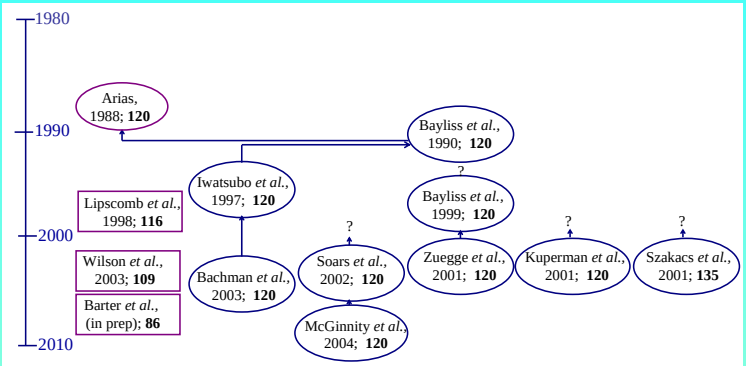

Figure 1: An overview of mean values of HPGL (x 10^6 cells g^{-1}) reported for humans (studies determining values experimentally are indicated by rectangles, those that cite values from other studies or where source and/or value used are unclear are indicated by ovals).

MATERIALS & METHODS

- In order to calculate the number of hepatocytes accurately in a given mass of liver, corrections must be made for the incomplete yield of cells following perfusion of the liver sample (Figure 2).
- Values of HPGL (n=24) were determined after accounting for the fractional loss of hepatocytes during processing. Fractional loss was corrected by measurement of the hepatocyte specific marker, CYP450 in homogenates prepared from matched liver tissue and hepatocyte suspensions [2] (Figure 3).
- Repeated measurements (n=3) of the liver samples allowed separation of experimental variability and thus estimation of inter-individual variability in HPGL (ANOVA).

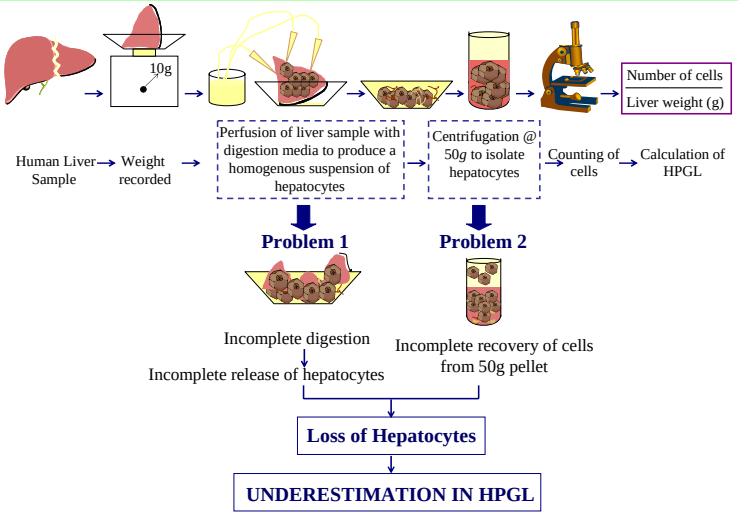

Figure 2: Schematic representation of hepatocyte isolation and calculation of HPGL. Two potential causes of error in HPGL determination are highlighted.

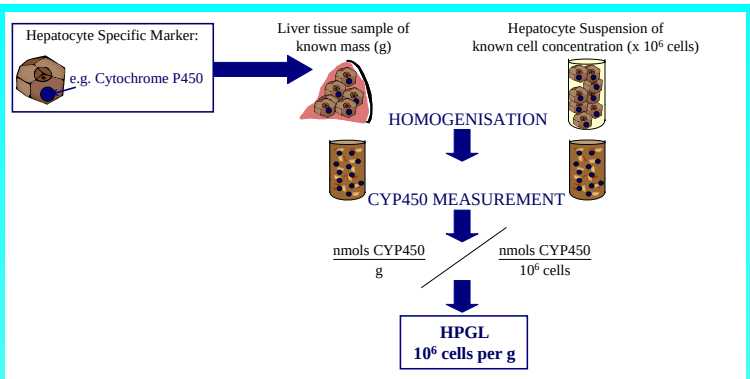

Figure 3: Use of a hepatocyte specific marker to correct for loss of cells during hepatocyte isolation.

RESULTS

- The geometric mean value of HPGL was 86 x 10^6 cells.g^{-1} (95% CI $mean_{geo}$: 72 – 102 x 10^6 cells.g^{-1}; 95% CI of observations: 36 – 201 x 10^6 cells.g^{-1}).
- Inter-individual differences in HPGL between livers were significant (ANOVA $p < 0.05$) with values ranging from 35 – 184 x 10^6 cells.g^{-1} (Figure 4).
- No relationship was found between HPGL and donor sex, smoking or drinking habits ($p > 0.05$).
- However, donor age was found to be a significant covariate of HPGL, with values decreasing with age ($p = 0.018$) (Figure 5).

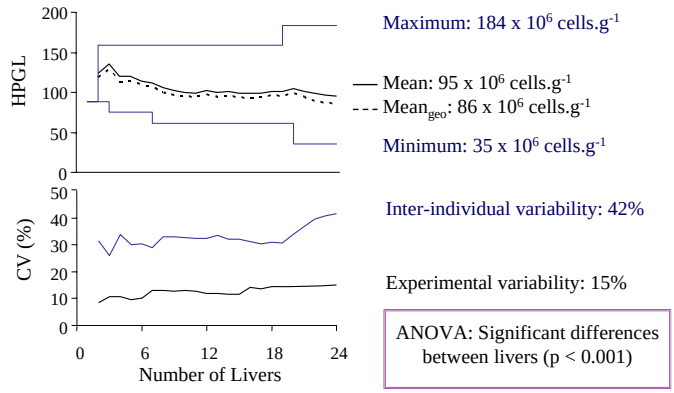

Figure 4: (a) Mean and $Mean_{geo}$ values of HPGL over the course of the study.
(b) Inter-individual and Experimental variability in values of HPGL over the course of the study.

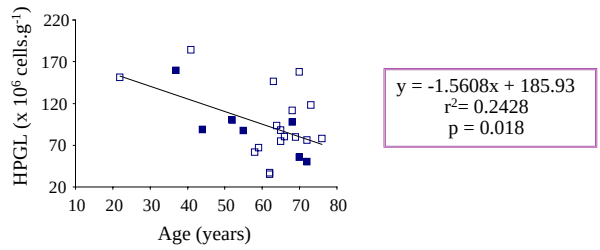

Figure 5: Relationship between HPGL and liver donor age; female (n = 7) (■), male (n = 17) (□) .

CONCLUSIONS

The findings provide more accurate estimates of population mean and inter-individual variability of a key scaling factor used in IVIVE.

REFERENCES

[1] Arias *et al.*, (1988) *The Liver Biology and Pathobiology* p9
[2] Wilson *et al.*, (2003) *Br J Clin Pharmacol* 56: 433