

Supplementary Material

Comparative use of aqueous humour ¹H NMR metabolomics and potassium concentration for PMI estimation in an animal model

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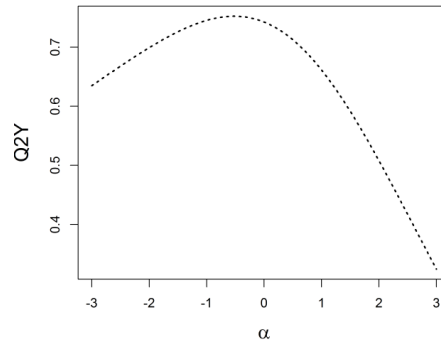


Fig. S1 Regression model of PMI vs $[K^+]$: plot of Q^2Y vs α used to estimate the optimal value of the power α

$[K^+]$ and AH metabolite concentrations vs PMI

To evaluate whether the combination of $[K^+]$ and AH metabolite concentration can improve the performance of the model of PMI obtained considering only the AH metabolite concentration, oCPLS2 was applied to model PMI according to eq. 3.

oCPLS2 is a regression technique that allows the inclusion of constraints in the framework of PLS. Specifically, the matrix of the constraints was set codifying the qualitative factor open/closed eye as two dummy variables with 0 and 1 depending on the state of the eye. Three scaling factors were considered: Pareto scaling, unit variance scaling and no scaling. Data were mean-centred. The number of latent variables and the scaling factor β were determined maximizing Q^2Y . In Fig. S2 the global SDEP obtained predicting the test set is reported for the models obtained assuming a β between 0 and 3, and an α between -3 and 3.

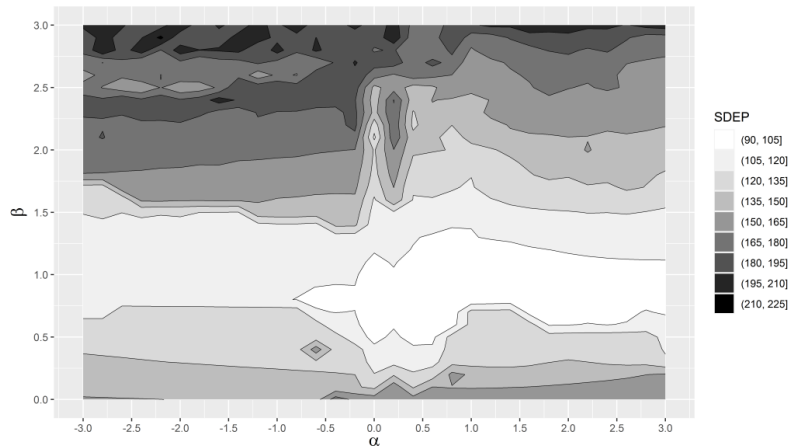


Fig. S2 Multivariate regression models based on oCPLS2 where both $[K^+]$ and AH metabolite concentrations are used to estimate PMI. The contour plot shows the behaviour of the global SDEP with respect the parameters β and α

[K⁺] vs AH metabolite concentrations

To investigate the relationships between [K⁺] and AH metabolite concentration ptPLS2 was applied with the objective of generating a multivariate calibration model.

Three scaling factors were considered: Pareto scaling, unit variance scaling and no scaling. Data were mean-centred. The number of latent variables and the scaling were determined maximizing Q²Y. As reported in Fig. S3a, the total explained variance of [K⁺]^α was greater than 0.60 for all the models with α in [-3, 3] and the fraction of the total variance of the X-block of the metabolites used to model the response was approximately equal to 22% independently of α. Moreover, the unexplained variance of [K⁺]^α, which is orthogonal to the block of the metabolites, showed a poor correlation with PMI for all the values of α (Fig. S3b).

These results proved that the variation in [K⁺]^α useful to explain PMI can be modelled by a linear combination of the metabolite concentrations and that [K⁺]^α should not add information when it is coupled with the data set composed of the AH metabolites (as previously observed).

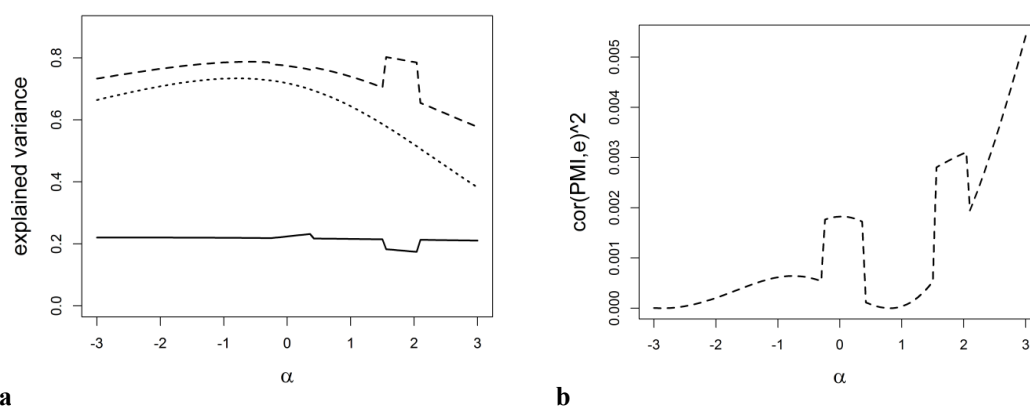


Fig. S3 Investigation of the relationships between [K⁺] and AH metabolite concentrations. Panel A: ptPLS2 models considering α between -3 and 3. The dashed line indicates R^2Y , the dotted line the best Q^2Y and the black line the fraction of the total variance of the X-block used to explain [K⁺]. Panel B: square of the Pearson correlation coefficient of PMI and the residuals of [K⁺] (i.e., the term e in eq. 4) vs α