

Use of metabolomics to find markers of zinc status in plasma and urine from Peruvian infants

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Introduction

- Zinc deficiency is associated with poor growth, depressed immune function, and increased susceptibility to and severity of infections
- Dietary requirements are estimated by factorial analysis using data on absorption and endogenous losses; there are no reliable biomarkers of zinc status
- Our aim was to assess whether changes in dietary zinc intake could be detected in plasma and urine samples by proton NMR and, if so, to identify possible novel biomarkers of zinc status

Experimental

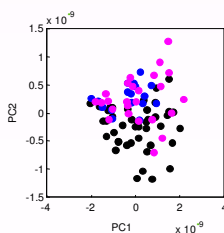
Design

- 6-8 month old Peruvian children were randomly assigned to receive iron-fortified cereal porridge and a separate multi-vitamin supplement with or without 3mg zinc for 6 months
- Plasma (101 individuals) and urine (43 individuals) samples were collected at the beginning and end of the intervention. Zinc concentration was measured by ICP-MS (Agilent 7500C, Palo Alto, CA). ¹H NMR spectra (600 MHz, Bruker) of plasma and urine extracts were recorded

Results

1. Growth effects

- Initial and final stages were distinguishable from both plasma and urine ¹H NMR spectra (Figure 1)
- Plasma lactate and an unknown metabolite decreased in both groups but no further discrimination was observed in the post-intervention samples (not shown)
- As anticipated¹, urinary betaine decreased, as well as glycine and citric acid. Hippuric acid slightly increased. The latter could reflect the children's diet diversification and the development of their gut flora as hippuric acid is believed to be the end product of certain dietary nutrients metabolized by gut bacteria



- Initial "control" + "added zinc"
- Final "control"
- Final "added zinc"

Figure 1. PCA on NMR spectra from urines at the beginning and the end of the intervention

2. Variability among human profiles

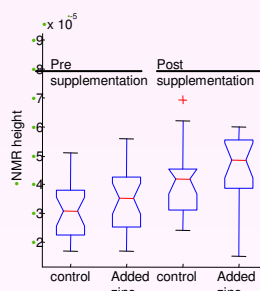


Figure 2. Peak height distribution of a signal at 1.08 ppm for the control and "added zinc" groups

- A genetic algorithm (GA) differentiated the final urine samples from the two groups due to a doublet at 1.08 ppm. Unfortunately, the technique also separated the baseline samples (Figure 2). This means that there was an initial difference between the two groups, although they were randomly assigned to the two interventions

- This is not the first time that this marker has been reported to differentiate human urine profiles. Slight variations in levels of a few metabolites are responsible for differentiating urinary profiles of individuals (Le Gall, unpublished)

3. Correlation between zinc levels and NMR plasma spectra

- Plasma zinc concentrations were correlated to NMR plasma spectra by PLS-Multivariate linear regression (Figure 3)

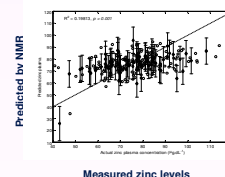


Figure 3. Measured zinc levels versus predicted by NMR. PLS model based on 771 variates and 4 PLS factors. open symbols, cross-validated predictions; solid symbols, test set predictions

- The correlation coefficient ($r=0.19$) was weak but significant ($p<0.001$)

- The variates selected to produce the best regression model highlighted signals from lipoproteins and choline related compounds (Figure 4)

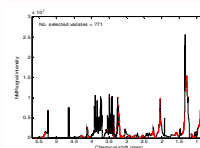


Figure 4. Mean NMR spectra of the fingerprint region with the variates included in the PLS model marked in red

Conclusions

- Markers for children's growth were identified in both urine and plasma, with clearer effects for urine
- NMR plasma spectra, in particular the regions associated with lipidic entities, appeared to correlate with measured plasma zinc concentration
- It was not possible to differentiate between the two groups post-intervention
- The increase in plasma zinc in the group receiving zinc was subtle ($+3 \text{ ug.dL}^{-1}$)². It is possible that ¹H NMR sensitivity and/or the study were not adequate to detect potential markers

ACKNOWLEDGMENTS This work was funded by the UK BBSRC and the Bill & Melinda Gates Foundation

¹ Prog. Nucl. Mag. Res. Sp. 1989;21, 449-501 ² Am J Clin Nutr 2007;85:538-47