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A Novel Spectrophotometric Approach for the Measurement of Submicromolar Concentrations of Nitrite and Nitrate in Biological Fluids

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Nitric oxide (NO) is an important mediator. Alteration in NO production has been implicated in a number of clinical conditions including renal failure. NO has a short half-life and therefore it is difficult to directly assess its concentrations body fluids. Hence, nitrite and/or nitrate levels are assessed as surrogate biomarkers of total NO production in the human body. A variety of analytical methods (i.e. spectrophotometric, spectroflourometric, HPLC-coupled to spectrophotometric- or spectroflourometric detectors, HPLC-MS and GC-MS have been developed for the measurement of NO metabolites. Of these, Griess-reaction is the most widely employed. The aims of present study were to develop: 1) an improved protocol for the preparation of serum nitrite and total nitrite (sum of nitrite + nitrate) by generating a genuine blank for every individual sample; 2) optimizing the condition for protein removal by $ZnSO_4$; and 3) exploring the effect of residual Cd, Cu and Zn on signal detection following the Griess reaction.

Recipient of first kidney [n=20] were recruited. Serum sample collection started collected at day one pre-transplantation and continued up to 2 months post-transplantation (i.e. 20 separate time points). Sample preparation involved nitrate reduction employing a mixture of Cd/Cu as catalyst, protein removal, performance of the Griess reaction and UV measurement at 545 nm.

It was found that the medians for nitrite and total nitrite levels in the candidates for renal transplantation were 0.6 μM and 1 μM, respectively. Three days after kidney transplantation, the corresponding levels dropped to 0.3 μM and 0.4 μM and remained fairly unchanged during the course of investigation.

In conclusion, this investigation has revealed that total nitrite concentration in patients with end stage renal failure is approximately 1 μM which is markedly lower than those data previously by other investigators using similar techniques. Our data also implies that final spectrophotmetric measurement is influenced by the protocol for protein removal as well as the presence of residual Cd and Cu. These findings may explain the large differences in reported values for above-mentioned NO metabolites in health and disease.