

## Letter to the Editor

### Nitric oxide synthesis in chronic renal failure. Are plasma *S*-nitrosothiol levels elevated?

To the Editors:

Under physiological conditions, less than 1% of L-arginine is converted to nitric oxide (NO) and L-citrulline in humans by the action of the NO synthase (NOS) family. Nevertheless, the L-arginine/NO pathway actively and potently participates in the regulation of multiple vital biological functions including regulation of vascular tone. NO and NO species containing physiological compounds, especially *S*-nitrosothiols, develop their biological actions via cGMP-dependent and cGMP-independent mechanisms.

Characterization of the L-arginine/NO pathway status in humans on a quantitative basis is a prerequisite for possible therapeutic intervention and represents a great scientific challenge. Authentic NO in the circulation is not accessible to analytical assessment because of its very short half life and its very low concentration. This methodological difficulty can satisfactorily be circumvented by quantifying NO derivatives in blood or urine which are accessible to analytical assessment and reliably reflect NO synthesis in humans. Circulating and urinary nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ), the major oxidative products of NO, have been recognized as reliable quantitative indicators of whole body NO synthesis. On the basis of  $\text{NO}_2^-/\text{NO}_3^-$  levels measured in plasma and/or urine, several diseases have been identified in which the status of the L-arginine/NO pathway is altered in comparison with that in health.

Unlike  $\text{NO}_2^-$  and  $\text{NO}_3^-$ , the significance of other NO derivatives, especially of *S*-nitrosothiols and 3-nitrotyrosine, is largely uncertain. Many factors have contributed to this uncertainty so far. In our opinion [1,2], insufficient attention has been paid to analytical chemistry in the field of NO research. A wide spec-

trum of analytical approaches and methods have been developed and applied for the quantitative determination of physiological *S*-nitrosothiols. These methods have yielded highly divergent plasma levels, often within a range of three orders of magnitude and have led to deceptive conclusions [1].

In the study by Wlodek et al. [3], recently published in *Clinica Chimica Acta*, the authors reported on alteration in plasma levels of nonprotein reduced thiols,  $\text{NO}_2^-/\text{NO}_3^-$  and *S*-nitrosothiols in chronic renal failure (CRF) patients as compared with those in healthy subjects. We would like to comment on this study, especially emphasizing the great differences regarding plasma levels of *S*-nitrosothiols in healthy subjects and CRF patients measured by this and other groups. Furthermore, the authors present their work on the premise that uremia is a state of elevated NO synthesis, although there is convincing evidence for the opposite.

In plasma of healthy subjects, Wlodek et al. [3] have measured  $\text{NO}_2^- + \text{NO}_3^-$  at a mean value of 120 nmol/ml, which is up to six times higher than literature values [4,5]. In plasma of CRF patients before hemodialysis, Wlodek et al. have measured  $\text{NO}_2^- + \text{NO}_3^-$  at 165 nmol/ml, which is increased by a factor of only 1.4 with respect to control. We and others have found plasma  $\text{NO}_2^- + \text{NO}_3^-$  levels enhanced by a factor of 1.9 (e.g., 36 nmol/ml in control vs. 69 nmol/ml in CRF [5]). Also, in plasma of healthy subjects, Wlodek et al. [3] have measured nonprotein sulfhydryl groups at a mean value of 87 nmol/l, which is 4.8 times higher than literature values [6].

Most importantly, however, Wlodek et al. [3] have reported *S*-nitrosothiol plasma levels of 8.8 nmol/ml in healthy subjects. These authors have determined *S*-nitrosothiol levels in plasma of their control and CRF groups using the fluorometric method of Marzinzig et al. [7], who have reported *S*-nitrosothiol plasma levels of only 0.45 nmol/ml in healthy subjects. Thus,

Wlodek et al. values [3] are approximately 20 times higher than those of Marzinzig et al. [7] and of many other groups including ours [1,5,8]. In their CRF patients, Wlodek et al. [3] have measured a mean plasma *S*-nitrosothiol level of 11 nmol/ml. This value is in full contradiction to the mean value of 0.15 nmol/ml measured by us in plasma of CRF patients ( $n=6$ ) for *S*-nitrosoalbumin, the major *S*-nitrosothiol in human plasma, which was comparable to that of healthy subjects (i.e., 0.18 nmol/ml,  $n=23$ ) [5]. The high divergence between the values of plasma levels of nonprotein reduced thiols,  $\text{NO}_2^- + \text{NO}_3^-$ , and especially of *S*-nitrosothiols in healthy humans reported by Wlodek et al. [3] and by the majority of other groups including ours [1,5,7,8], challenges the correctness of the measurements of Wlodek et al. [3] and doubts their conclusions.

The statement by Wlodek et al. [3] that CRF is accompanied by elevated NO synthesis is incorrect. There is convincing evidence that NO synthesis is decreased in CRF, most likely due to inhibition of NOS activity by endogenous inhibitors such as ADMA (i.e., asymmetric dimethylarginine), the plasma levels of which are elevated in renal failure [9–12]. There are numerous reports showing decreased NO production in hemodialysis patients [13,14]. Schmidt et al. have shown that, under a controlled low-nitrate diet,  $\text{NO}_2^- + \text{NO}_3^-$  output was significantly reduced in hemodialysis patients as compared to healthy controls [13]. These authors proved that to be true in CRF patients not on dialysis [14]. Therefore, decreased but not elevated NO synthesis in uremia seems to be causing the highly elevated cardiovascular risk in this disease. Decreased NO synthesis in uremia also could be responsible for the hypertension in this disease.

*S*-Nitrosothiols are potent vasodilators similar to NO and because of their very low levels it is unlikely that these compounds serve as antioxidants. Their roles in chronic renal failure, in other diseases and in health remain to be established. For this purpose, use of reliable analytical methods is absolutely required.

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6 June 2003

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