Letter to the Editors

Comments on 'Vascular effects of dietary nitrate (as found in green leafy vegetables and beetroot) via the nitrate-nitrite-nitric oxide pathway'

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It was our pleasure to read an excellent review by Lidder & Webb about the nitrate-nitrite-nitric oxide pathway [1]. These authors summarized the whole spectrum of nitrite and nitrate effects and provided a review of clinical trials using dietary nitrate interventions as well as suggesting nitrite/nitrate dose standardization.

We would like to comment on the nitrite effect on platelet function. In this part, the older reports cited by Lidder & Webb show that nitrite addition to platelet preparations had no effect on platelet function. We recently reported [2] that this is correct when only platelet rich plasma or similar pure platelet preparations are used. However, we showed that addition of red blood cells to such preparations combined with hypoxia had a significant effect on platelet functioning and inhibition of platelet aggregation was observed upon nitrite addition. These data are in agreement with the hypothesis that added nitrite is reduced to nitric oxide by hypoxic red blood cells and with our recent observation of altered platelet aggregation by dietary nitrate manipulation – mice fed a low nitrate diet had better platelet aggregation than mice on a standard diet (Park JW, unpublished data). This could be an important start for future investigations in clinical settings when enhanced platelet aggregation is either required or to be avoided.

We would also like to address one other point. Since neuroglobin and cytoglobin were discovered, there has been an intensive search for their biological functions. Neuroglobin is normally expressed in brain at very low levels and both globins are upregulated during hypoxia. They are able to bind oxygen, as well as CO and NO, so proposals for their function range from delivering oxygen to detoxifying deleterious NO during reperfusion.

Several recent papers cited by Lidder & Webb claim that neuroglobin is a nitrite reductase [3–5]. We question the physiological relevance of this pathway based on following considerations (i) Heme in neuroglobin exists either as 5-coordinated or 6-coordinated molecule. In cells

the equilibrium is shifted strongly towards 6-coordinated conformation with the distal histidine bound to heme at the position available for binding of other ligands when neuroglobin is in 5-coordinated state. This leads to a small fraction of the total neuroglobin to be possibly active at physiological conditions. All three cited papers used non-physiological conditions to detect nitrite reductase activity and reactions were carried out anaerobically. (ii) Only ferrous heme is able to reduce nitrite to NO. It is known that heme in neuroglobin oxidizes to the ferric state fairly easily and there have been unsuccessful attempts to find a neuroglobin-associated reductase that would reduce Fe back to its ferrous state [see [6] as example]. Similar considerations as explained in (i) and (ii) apply to nitrite reductase activity of cytoglobin under near-anoxic conditions recently described in cultured human aortic smooth muscle cells [7]. (iii) Even with these limitations it was necessary to introduce arbitrary structural mutations into neuroglobin to achieve significant levels of nitrite reduction [3, 4]. For these reasons, together with the low abundance of neuroglobin and cytoglobin, we believe that their function as nitrite reductases *in vivo* is physiologically irrelevant.

Competing Interests

All authors have completed the Unified Competing Interest form (available on request from the corresponding author) and declare BP and ANS had support from NIH for the submitted work; BP and ANS have been employed by NIH in the previous 3 years and ANS is a co-inventor of a patent issued to NIH for the use of sodium nitrite in the treatment of cardiovascular diseases.

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RECEIVED

13 September 2012

ACCEPTED

27 September 2012

ACCEPTED ARTICLE PUBLISHED ONLINE

19 October 2012

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