Letter to the Editors

Reply to 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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We thank Piknova & Schechter for their helpful comments [1], and for highlighting their recent important findings on the action of nitrite on platelet function [2], which we fully support. Unfortunately this paper was not included, as it was published around the time of submission of our review [3]. Our intention was to portray how nitrite had originally been dismissed as an inhibitor of platelet aggregation. In earlier studies, neither nitrosothiols nor nitrite were detected in plasma following nitrate ingestion and were thus considered unlikely mediators of the inhibitory effects of dietary nitrate on platelet function [4–6]. Also, the direct administration of intra-peritoneal nitrite actually increased (or had no effect on) *ex vivo* platelet aggregation in rats [7].

The discovery that nitrite inhibited platelet aggregation and accounted for the antiplatelet effect of dietary nitrate was made through our 'spitting experiments', which interrupted the conversion of nitrate (derived from beetroot juice) to nitrite via the enterosalivary circulation, blocking both the rise in plasma nitrite and the inhibition of platelet aggregation [8]. This surprising finding was supported by our demonstration of proof of concept by spiking *ex vivo* whole human blood with sodium nitrite, which inhibited aggregation, an effect not seen with nitrite spiking of platelet-rich plasma [9]. The understanding of these mechanisms has now been considerably extended by Srihirun *et al.* [2], who demonstrated the hypoxia-dependency of this erythrocyte-mediated process.

Piknova & Schechter also suggest that neuroglobin and cytoglobin are unlikely to be important nitrite reductases as they are only present in low concentrations, and only appear to possess this activity under anaerobic conditions with high concentrations of nitrite [10–13]. We had included these enzymes in our review for completeness, and since some very scientifically elegant and interesting mechanistic studies have been performed, particularly concerning the regulation of and reactions with the 6- and

5-coordinated heme pocket forms of neuroglobin [10] and the potential role of cytoglobin as an oxygen-dependent regulator of nitric oxide decay [14]. Indeed, most of the nitrite reductases are dependent on hypoxia and acidosis. However, we agree that, in the context of the vascular effects of dietary nitrate, it seems unlikely that neuroglobin and cytoglobin are major enzymes regulating the nitrate-nitrite-nitric oxide pathway under normal physiological conditions.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted work, and no other relationships or activities that could appear to have influenced the submitted work.

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