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PERSPECTIVES

To clot or not to clot? That is a free radical question

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Unravelling the biological roles of reactive oxygen species (ROS) is challenging (Murphy *et al.* 2011). There are three key challenges: (1) the global term 'ROS' subsumes chemically diverse free radical and non-radical species making it difficult to attribute a particular effect to a particular species; (2) nutritional antioxidants often fail to react appreciably with key ROS (e.g. superoxide anion, $O_2^{\cdot-}$) at the desired time and place; and (3) measuring ROS is a perennial difficulty because their short half-lives (usually in the order of milliseconds) can preclude direct detection (Murphy *et al.* 2011). It is unsurprising, therefore, that few suspected biological roles of ROS have been confirmed in humans, especially in an exercise context that presents its own unique experimental challenges. For example, debate still exists as to whether ROS regulate key adaptive responses to exercise (Margaritelis *et al.* 2016).

ROS may regulate coagulation (Gorlach, 2005), a critical blood clotting process (i.e. haemostasis) that is important in health and disease. A regulatory role for ROS largely rests on *in vitro* evidence, which suggests that the $O_2^{\cdot-}$ producing enzyme NADPH oxidase triggers generation of thrombin – a serine protease – by activating tissue factor (Gorlach, 2005). After the inactive zymogen pro-thrombin is converted to thrombin by tissue factor it promotes clotting by converting soluble fibrinogen to insoluble fibrin (Gorlach, 2005). It seems, therefore, that ROS activate haemostasis. Given the predominately *in vitro* focus, a critical gap in knowledge is whether ROS regulate haemostasis in the exercising human situation. Exercise disrupts redox homeostasis (Margaritelis *et al.* 2016), making it an excellent model to determine if haemostasis is redox regulated (Margaritelis *et al.* 2016). In an article in this issue of *The Journal of Physiology*, Fall *et al.* (2018) provide novel insight regarding the redox regulation of haemostasis *in vivo* in the exercising human.

To investigate the redox regulation of haemostasis, Fall *et al.* (2018) recruited 40 young healthy males and deployed a double-blind placebo-controlled nutritional antioxidant experimental design. The experimental design included three phases: (1) passive baseline testing in normoxia followed by 8 weeks of vitamin C (VC) and vitamin E (VE) supplementation; (2) post-supplementation involving repeated baseline testing; and (3) a redox challenge phase consisting of passive hypoxia for 6 h at 12% O_2 before active hypoxia wherein participants completed an incremental exercise test at 12% O_2. Importantly, the authors checked the participants had sufficient VC and VE before the supplementation period to exclude the possibility that their results were attributable to simply correcting dietary deficiency. Using passive and active hypoxia is a commendable experimental strategy because it provided two established ways of disrupting redox homeostasis by inducing systemic free radicals, enabling the authors to robustly test their hypotheses. During each phase, the authors took venous blood samples to assess haemostasis biomarkers, including thrombin–anti-thrombin complex and fibrinogen.

When using VC and VE it is imperative to confirm that they acted as antioxidants with supporting biochemical evidence (Murphy *et al.* 2011; Cobley *et al.* 2015; Margaritelis *et al.* 2016). To do so, the authors deployed electron paramagnetic resonance (EPR) spectrometry to unambiguously identify the VC radical. The VC radical is formed when VC donates an electron to a sufficiently reactive free radical (e.g. the hydroxyl radical), VE radical or a redox-active transition metal (Cobley *et al.* 2015). After confirming that supplementation increased circulating VC and VE content, the authors (Fall *et al.* 2018) showed that systemic VC radical abundance decreased post-supplementation, which is compatible with a decrease in systemic free radicals. Unexpectedly, VC and VE supplementation increased basal thrombin generation post-supplementation, implying that the decrease in systemic free radicals activated haemostasis, which is contrary to the authors' original working hypothesis that free radicals activate coagulation.

The results of the next phase of the study strengthen the association between a decrease in systemic free radicals and haemostasis. Specifically, disrupting redox homeostasis using passive hypoxia and active hypoxia curtailed thrombin generation in the supplemented group. That is, a probable increase in systemic free radicals, as supported by an increase in the VC radical, restored thrombin levels (Fall *et al.* 2018). The authors suggest that systemic free radical production is a hormetic phenomenon that contributes to physiological vascular haemostasis. However, dysregulated redox homeostasis contributes to vascular disease, so it will be important to disambiguate the boundary between physiological and pathological redox stress.

From a mechanistic perspective, how VC or VE acted to modify the redox environment is unclear because the VC radical reports on VC donating an electron, so it fails to disclose the identity of the reactant(s). Using *ex vivo* EPR-based spin trapping to measure free radicals would have helped interpret the VC radical data, with the caveat that resolving the identity of the free radical measured is still challenging. In considering $O_2^{\cdot-}$, VE fails to react appreciably and VC does react but is still likely to be outcompeted by extracellular superoxide dismutase on kinetic grounds, which is why $O_2^{\cdot-}$ is extremely difficult to detect directly (Cobley *et al.* 2015). Neither vitamin reacts appreciably with hydrogen peroxide, a non-radical species that can transduce redox signals. Perhaps, VC and VE acted synergistically to scavenge alkoxyl and peroxyl radicals because lipid peroxidation products can signal (Cobley *et al.* 2015); addressing their haemostatic role represents fertile ground for future investigations.

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As a wider concluding perspective, the new, unexpected result that systemic free radicals appear to regulate haemostasis in humans *in vivo* questions the practice of taking VC/VE supplements – unless one is deficient, they may do more harm than good.

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Additional information

Competing interests

None declared.

Author contributions

All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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