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SHORT COMMUNICATION

A red orange extract modulates the vascular response to a recreational dive: a pilot study on the effect of anthocyanins on the physiological consequences of scuba diving

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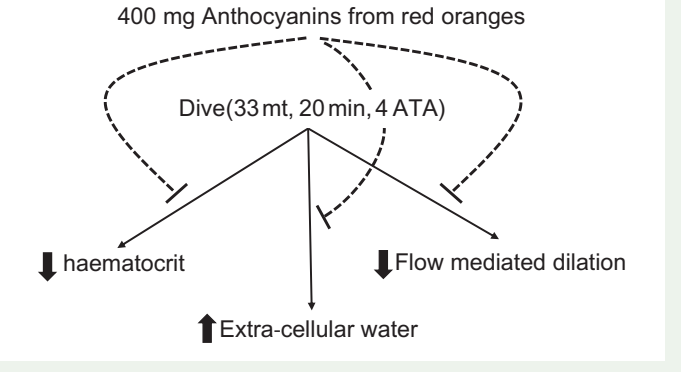
ABSTRACT

Nutritional antioxidants have been proposed as an expedient strategy to counter the potentially deleterious effects of scuba diving on endothelial function, flow-mediated dilation (FMD) and heart function. Sixteen volunteers performing a single standard dive (20 min at 33 m) according to US Navy diving procedures were randomly assigned to two groups: one was administered with two doses of 200 mg of an anthocyanins (AC)-rich extract from red oranges, 12 and 4 h before diving. Anthocyanins supplementation significantly modulated the effects of diving on haematocrit, body water distribution and FMD. AC administration significantly reduces the potentially harmful endothelial effects of a recreational single dive. The lack of any significant effect on the most common markers of plasma antioxidant capacity suggests that the mechanism underlying this protective activity is independent of the putative antioxidant effect of AC and possibly involves cellular signalling modulation of the response to high oxygen.



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1. Introduction

Recreational diving is a very popular outdoor sport activity, with millions of recreational divers worldwide. During dives, the breathing at increased oxygen and nitrogen pressure with compressed air imposes a specific stress to divers has been reported to affect haemodynamics and cardiovascular (CV) function. Scuba diving induces a complex set of responses associated with the direct effect of increased pressure on the body and of the increased flux of inhaled air (Wang et al. 2015). While the consequences of intravascular nitrogen bubbles formation have been largely addressed by researchers (Vann et al. 2009), the physiological response to increased oxygen flux is still scarcely known and understood (Perovic et al. 2014). An increase in free radicals and, in general, of reactive oxygen and nitrogen species has been frequently proposed as the causative event leading to the physiological response to diving and eventually to endothelial dysfunction (Obad, Palada et al. 2007). Accordingly, nutritional antioxidants (Vitamins C and E) have been reported to attenuate, but not fully prevent, acute arterial endothelial dysfunction after diving (Obad, Valic et al. 2007). The consumption of dark chocolate, rich in polyphenols, owing a putative antioxidant activity has been shown to counter, at least in part, endothelial dysfunction induced by SCUBA and breath-holding diving (Theunissen, Guerrero et al. 2013; Theunissen, Schumacker et al. 2013; Theunissen et al. 2015).

More recently, more complex pathophysiological mechanisms underlying CV response to diving have been proposed, involving the expression and activity of specific proteins (sirtuins – SIRTs) implicated in the modulation of a wide range of cellular processes, including stress resistance, as well as energy efficiency and alertness during low-calorie situations (Corbi et al. 2013). Polyphenols, and in particular anthocyanins (AC), have been reported to modulate the expression and activity of several enzymes involved in nitric oxide (NO) metabolism and to elicit an adaptive response involving the transcription factors HIF and Nrf2 (Cimino et al. 2013). More specifically, AC from red orange have been reported to exert a wide spectrum of activities including the modulation of cell signalling (Speciale et al. 2014) and the control of energy metabolism (Cardile et al. 2015).

According to this evidence, and on the basis of our previous published experience (Theunissen, Schumaker et al. 2013), we have hypothesised the possibility that AC administration could modulate some of the endothelial responses to high oxygen associated with diving.

This article reports the results of a preliminary pilot study addressing the effects of the administration of an AC-rich extract from red oranges on some specific hemodynamic parameters. Haematocrit (HTC), body water distribution and flow-mediated dilation (FMD) have been assessed in humans before and after a single underwater dive at recreational level, 20 min at 33 m of freshwater (roughly 4 ATA) performed in an indoor controlled environment, within the no decompression limits according to the US Navy Tables (see http://www.ndc.noaa.gov/).

2. Results and discussion

2.1. HTC and body hydration

AC administration had no effect on pre-dive HTC value (not shown). Overall, dive was associated with modest changes in HTC and body hydration. However, at the end of the dive, a small

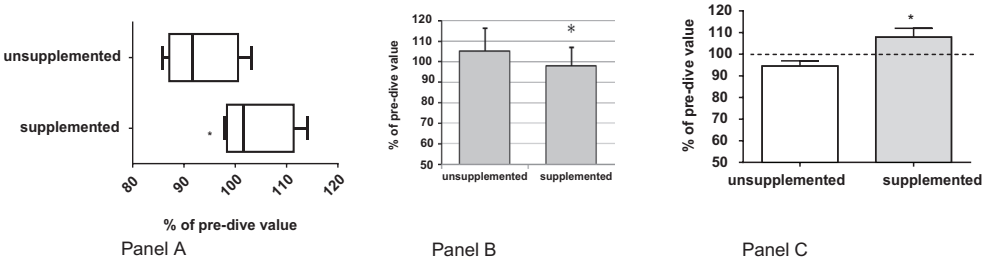

Figure 1. Panel A: HTC changes (as % of pre-dive value) after diving and effect of the administration of 400 mg ROC. Panel B: Extracellular water measured by multifrequency bioimpedance changes (as % of pre-dive value) after diving and effect of the administration of 400 mg ROC. Panel C: FMD changes (as % of the initial value) after diving and effect of the administration of 400 mg ROC. *$p < 0.05$ according to Mann–Whitney *U*-test vs. unsupplemented.

but significant ($p < 0.05$) difference of HTC was observed between non supplemented divers and subjects administered with ROC (Figure 1, Panel A). This observation was unexpected, since it is known that divers referring to hyperbaric centres for decompression sickness treatment usually present a significant haemoconcentration. This observation is in agreement with the reported increase in diuresis, natriuresis, kaliuresis and in free water clearance, eventually compensated by a subsequent shift from extracellular space to plasma in the attempt of the organism to recover from hypovolaemia and accompanied by thirst-inducing osmoregulatory signals (Pendergast & Lundgren 2009). We can hypothesise that the dive induces a fluid shift to compensate immersion-induced diuresis. This water transfer would be a 'temporary loan' from cellular to vascular compartment. However, available data show that a reversal of this fluid shift occurs at some later point after the dive (Gempp et al. 2009).

The ability of AC in buffering HTC changes indicates that alterations of the ratio between extracellular water to intracellular (ECW/ICW), associated with a temporary increase in plasma volume, occur immediately after surfacing and that this event can also be significantly affected by AC administration. This study does not provide any element to understand this effect, which is likely to be due to an either direct or indirect interaction with the ability to maintain osmotic pressure.

Body impedance analysis indicates that a small increase in extracellular water as measured by the resistance values at 5 kHz occurs in control divers, which is completely reversed by a pre-dive administration of ROC (Figure 1, Panel B). The figure reports raw resistance data, as we considered that available algorithms to convert impedance to total body water and body water compartmentation (either published in the available literature or provided by the manufacturer) could be invalidated by electrolytes shift due to the dive. However, this change is supposed to be significantly associated with an ECW shift (Sengun et al. 2012). Values obtained at 50 and 100 kHz were not significantly affected by diving in both groups.

2.2. Flow mediated dilation

Previous studies reported that scuba diving is associated with a temporary significant alteration of vascular dynamics (Theunissen, Guerrero et al. 2013). In this study, after surfacing, unsupplemented and AC supplemented divers obtained an average score at FMD test about 5% lower and 6% higher, respectively, than before diving. Even though the within-group

difference of pre-dive values was not significant, it is interesting to note that post-dive values of divers receiving AC were significantly higher than those observed in control subjects (Figure 1, Panel C). This observation is in agreement with published studies (Rodriguez-Mateos et al. 2013) reporting a significant protective effect of AC and other nutritional antioxidants (Obad, Valic et al. 2007; Theunissen, Guerrero et al. 2013; Theunissen et al. 2015) towards endothelial dysfunctions and in particular in the modulation of nitric oxide-mediated vascular responses.

2.3. Discussion and conclusion

The pilot study presented herein was conducted in order to find a preliminary field confirmation in 'real condition' of the observation obtained at molecular level *in vitro* indicating that polyphenols, and in particular AC, modulate the cellular response to pulsed hyperoxia in cultured primary human endothelial cells (Cimino et al. 2012, 2013). In fact, reports addressing this hypothesis *in vivo* are still scarce (Vukovic et al. 2009).

Our data corroborate our previous studies that demonstrated that temporary moderate hyperoxia is associated with the unexpected activation of hypoxia inducible factor-1 (HIF-1) and to the activation of the hormetic transcription factor nuclear factor erythroid-2 like (Nrf-2) (Cimino et al. 2012). These two transcription factors have apparently distinct mechanisms of activation, both related to fluctuation of oxygen availability and both converging to the expression of genes involved in cellular survival and recovery from stress, and both control the expression of genes involved in endothelial function (Miyata et al. 2013).

The hypothesis of an interplay between HIF-1 and Nrf-2 provides a conceptual background for the understanding of the activity of AC in countering the effects of high-pressure oxygen breathing on endothelial functions, independently on their putative antioxidant (chain breaking) capacity. In fact, the biological activity of AC (and in general of nutritional phenolics) has been recently revisited under an perspective independent of a *bona fide* antioxidant properties (because of evident stoichiometry limitation *in vivo*), in favour of the capacity to activate Nrf-2 to bind to the antioxidant response element/electrophile response element (ARE/EpRE) in the promoter of genes encoding for 'protective' proteins (Forman et al. 2014). Interestingly, in our subjects, AC supplementation did not result in a significant increase in common markers of non-enzymatic antioxidant capacity (total radical-trapping antioxidant parameter-TRAP and ferric reducing antioxidant power-FRAP) and, similarly, no significant changes were observed after diving, neither in the group of supplemented divers nor in the un-supplemented ones (data not shown). This observation suggests that the administration of 400 mg ROC over 16 h in people undergoing a normal diet does not lead to a significant increase in plasma antioxidant parameters and that breathing compressed atmospheric air at about 4 ATA for 20 min is not associated with a significant effect on non-enzymatic plasma antioxidant values.

Besides hyperoxia, it has been recently shown that barometric pressure has direct effect on cells redox status (Wang et al. 2015). However, the effect of hydrostatic pressure and the subsequent diuresis or dehydration associated with the immersion is mainly hormone-related and if a direct pressure effect on the body or body cells was present, the same would account for both the supplemented and unsupplemented divers. Therefore, the differences observed suggest the presence of other mechanisms.

In this pilot study, AC supplementation was administered into two separate dosages: the first one at about 12 h from the dive and the second one at about 4 h before the immersion. The second administration was designed to induce a peak of maximum plasma concentration of anthocyanin metabolites at the time of immersion (Rodriguez-Mateos et al. 2013). Data collected let us speculate about the presence of two mechanisms induced by AC and affecting endothelial functions and water movements: one due to fast signals induced by the second ROC administration and possibly mediated by extracellular regulated kinase 1 and 2 (ERK1/2), and a second slower one (induced by the first ROC administration) due to the expression of genes involved in cellular response to high oxygen, both affecting nitric oxide bio-availability and vascular functions (Speciale et al. 2014).

According to this mechanism, further studies are needed to establish if nutritional flavonoid (and in particular AC) supplementation can be a standard strategy to be advised not only to recreational or professional divers, but also to people exposed to high oxygen, either for therapeutic purposes or accidental collateral events (Eftedal et al. 2013).

Disclosure statement

No potential conflict of interest was reported by the authors.

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