SUPPLEMENTARY MATERIAL

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.

16 volunteers performing a single standard dive (20 minutes at 33 m) according to US Navy diving procedures were randomly assigned to 2 groups: one was administered with two doses of 200 mg of an anthocyanins rich extract from red oranges, 12 hrs and 4 hrs before diving. Anthocyanins supplementation significantly modulated the effects of diving on Hematocrit, 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.

Keywords: diving, anthocyanins, endothelial dysfunction, Flow-mediated dilation, body water

Experimental section

Materials and methods

Dive profile and timeline of measurements

Details about the enrolment procedure have been reported elsewhere (Theunissen *et al.*, 2013). Briefly, 16 experienced (4 females and 12 males, aged 40.1 ± 5.8 years) scuba divers (minimum certification “Autonomous Divers” according to ISO 24801-2 with at least 50 logged dives) volunteered for this study. All subjects were non-smokers with regular, but not professional, physical activity. None of the subjects had a history of previous cardiac abnormalities, and none of them was under any cardio-active medication. None was taking either vitamin or “herbal supplements”. Dives, measurements and blood withdrawals were performed in a pool environment (Nemo33, Brussels, Belgium) just before and within 15 minutes after surfacing from the dive. This facility was chosen to mitigate confounding factors related to the dive itself, for instance, effort may affect nitrogen uptake at depth, underwater currents are often the cause of such effort, here very standardized conditions were met since no effort was needed to fight against current. Another very important issue is the temperature of the water, the pool has a constant temperature of 33 C°, very close to thermoneutral conditions adding to the standardization of conditions. Venous blood samples were drawn in EDTA tubes from the antecubital vein. Blood was centrifuged within 4 hours of their sampling, and serum stored at -40°C pending analysis.

Divers performed a dive to a depth of 33 meters for 20 minutes. This depth-time profile falls within accepted “no-decompression limits” according to the US Navy Tables (see <http://www.ndc.noaa.gov/>). Further details about the dive have been published elsewhere (Theunissen *et al.*, 2013). Divers were asked to avoid Sauna (Blatteau *et al.*, 2008), vibration mats (Germonpre *et al.*, 2009), or heavy exercise 24 hours before diving since these activities can interfere on the endothelial function as well as on the “decompression stress” by means of a denucleation process (Madden *et al.*, 2013).

Anthocyanin administration

Subjects were randomly assigned to two groups: one group was administered with two capsule of 200 mg of Red Orange Complex (ROC™), a proprietary AC rich extract from red oranges (Bio-NAP Ltd. Catania, Italy) and the second one was assigned to a placebo (glucose). More information about ROC are available at http://www.bionap.com/index.php?option=com_content&view=article&id=53&Itemid=64&lang=en. Capsules (ROC or placebo) were consumed at the dinner of the day before the dive and at breakfast about 4 hours prior the dive, respectively.

Endothelial function

Arterial endothelial function was assessed before and after a scuba dive by measuring the flow-mediated dilation (FMD) of the brachial artery as described elsewhere (Brubakk *et al.*, 2005). Briefly, FMD was measured with a 5-10 MHz transducer (Mindray dp 6600, Mindray, China) and optically controlled by a researcher. FMD was computed as the percentage change in brachial artery diameter measured at peak dilation by means of a boundary tracking software (FMd-I software, FloMedi, Belgium)

Haematocrit

Haematocrit was assessed in a routine automated manner using red laser light (ADVIA 2120, Siemens AG Healthcare Sector, Erlangen, Germany).

Body water distribution

Total body impedance was assessed at 5 kHz, 50 kHz, 250 kHz and 500 kHz frequencies as described elsewhere (Zhu *et al.*, 1998) by a Tanita MC-180 MA Multi-Frequency Body Composition Analyzer (Tanita Co., Ltd, Tokyo, Japan 174 -8630 Japan).

Statistical analysis

Statistical analyses were performed using GraphPad Prism 5 (La Jolla, CA, USA). Data are presented either as average values or as a percentage of pre-dive individual values. Differences were compared by *Wilcoxon-Mann-Whitney* and *Kruskal-Wallis* tests for non-parametric data sets. Statistical significance level was set *a priori* at $P < 0.05$.

References

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