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Comment on Hydroxytyrosol Induces Proliferation and Cytoprotection against Oxidative Injury in Vascular Endothelial Cells: Role of Nrf2 Activation and HO-1 Induction

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In their recent publication in this Journal, Zrelli et al.[1] describe the induction of proliferation and cytoprotection against oxidative injury by the olive oil constituent hydroxytyrosol (HT). The addition of HT in the range of 50−100 μM to porcine vascular endothelial cells (PVECs) increased cell proliferation, improved wound repair, and rendered cells partly resistant to H_2O_2-induced cytotoxicity. On the basis of subsequent experiments, the authors argue that HT up-regulates the antioxidant defense system in PVECs through the activation of Nrf2-controlled enzymes, in particular, heme oxygenase-1 (HO-1).

However, Zrelli et al., like many other researchers in the field, conducted their experiments without addressing (either experimentally or in the discussion) the possibility of HT-induced cell culture artifacts. In a series of experiments, it has recently been shown that HT and many other phenolic compounds added to standard cell culture media (such as DMEM, MEM, or RPMI) rapidly produce H_2O_2 in the one- to three-digit micromolar range.[2−5] In fact, we detected up to 100 μM H_2O_2 in DMEM supplemented with HT at concentrations used in the paper by Zrelli et al. (Figure 1A). As our initial studies were conducted in DMEM with high glucose concentrations and supplemented with 10% fetal bovine serum (FBS) and antibiotics,[3] we repeated the experiment with the cell culture medium composition given by Zrelli et al. (i.e., DMEM with low glucose concentrations and supplemented with 1% FBS and antibiotics). As we expected, significant amounts of H_2O_2 were generated in the low-glucose DMEM (Figure 1B), with absolute H_2O_2 levels being even higher than in our previous studies. The higher production of H_2O_2 could perhaps be due to differences in the amount of supplemented FBS (10% vs 1%) and not related to the medium's glucose content, as glucose is not known to interact with H_2O_2. Fabiani et al. have also shown that HT-generated H_2O_2 is able to induce apoptosis in HL60 cells.[4,5]

In contrast, H_2O_2 is known to stimulate the proliferation of various cell types at micromolar concentrations.[6] Sigaud et al.,[7] for example, found that chronic exposure of primary alveolar epithelial cells to a constant flux of 10 μM H_2O_2 (generated by a glucose/glucose oxidase system; G/GO) caused increased cell proliferation. Similar effects were reported by Ruiz-Ginés et al.[8] when using G/GO for producing low amounts of H_2O_2 in cultures of bovine aortic endothelial cells. Importantly, in the same study, the addition of a single bolus of H_2O_2 between 1 μM and 1 mM had no effect on cell proliferation. This observation suggests that pro-proliferative effects of H_2O_2 depend more on a constant flux of (comparatively low) concentrations of H_2O_2 than on the absolute amount of H_2O_2 added to a cell culture system. Our own data clearly show that H_2O_2 production in HT-supplemented cell culture media is maintained for at least

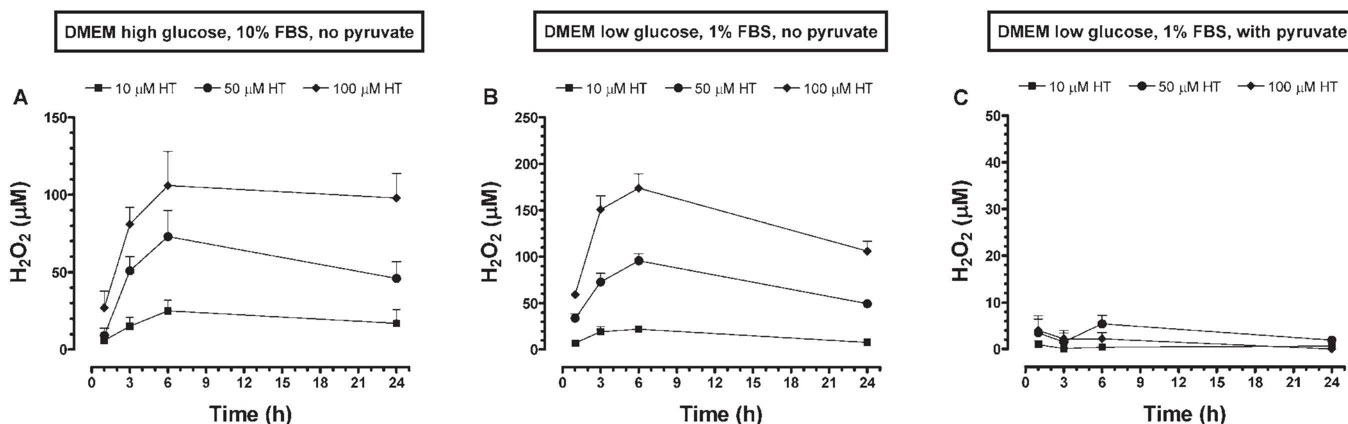

Figure 1. Time course of HT-induced production of H_2O_2 in DMEM medium: (A) DMEM with high glucose (4.5 g/L), supplemented with 10% FBS (data taken from ref 3); (B) DMEM with low glucose (1 g/L), supplemented with 1% FBS; (C) DMEM with low glucose (1 g/L), supplemented with 1% FBS and pyruvate (110 mg/L). The amount of H_2O_2 was quantified using the FOX assay according to the method given in ref 9. Data are the mean ± SD ($n \geq 3$).

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24 h.[3] Hence, the question must be asked as to whether the effects described by Zrelli et al. are due to direct effects of HT on PVECs or are perhaps the result of the artifactual production of biologically relevant amounts of H_2O_2 arising from either the interaction of HT with cell culture constituents or its autoxidation[5] or both. We have previously reported that HT breaks down substantially when added to cell culture medium,[3] suggesting that HT degradation products (with currently unexplored effects) might have also affected the PVECs given that cells were preincubated with HT (in most experiments for 24 h) prior to the actual experiment.

In addition, and as depicted in Figure 1C, the HT-induced accumulation of significant amounts of H_2O_2 also depends on the presence of pyruvate in the cell culture medium. Pyruvate readily removes H_2O_2 from cell culture media and is simultaneously destroyed, which can affect results when pyruvate is an important nutrient to the cells being cultured.[5,9,10] However, aside from the amounts of glucose, FBS, and antibiotics, Zrelli et al.[1] provided no further details regarding the composition of their DMEM, making it thus difficult to identify which medium has been used because the supplier, Sigma-Aldrich, offers various subtypes of DMEM low-glucose medium with and without pyruvate.

Consequently, we suggest that the conclusions drawn by Zrelli et al.[1] may need to be re-evaluated.

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