## SL09 Red wine polyphenols promote endothelial nitric oxide release by enhancing endothelial nitric oxide synthase expression

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Population based studies suggest a reduced incidence of morbidity and mortality from coronary heart disease by moderate and regular consumption of red wine (1). Endothelial nitric oxide (NO) is a pivotal vasoprotective molecule. In addition to its vasodilating feature, endothelial NO has anti-atherosclerotic properties by inhibiting platelet aggregation, leukocyte adhesion, smooth muscle cell proliferation and the expression of genes involved in atherogenesis (2). This study, therefore, examines the influence of red wine polyphenols on the regulation of eNOS expression and subsequent NO synthesis focusing at putative long-lasting anti-atherosclerotic effects of red wine. Treatment (20 h) of human umbilical vein endothelial cells (HUVECs) and of the HUVEC-derived cell line EA.hy926 with a alcohol-free red wine polyphenol extract (RWPE) led to a dose-dependent (100-600 µg/mL), significant increase in NO release (up to 3.0-fold/HUVEC and 2.0-fold/EA.hy926) by use of the fluorescent probe DAF-2 (4,5-diaminofluorescein). This effect was corroborated by the [$^{14}$C]L-arginine/L-citrulline conversion assay in intact EA.hy926 cells. RWPE (20 h, 100-600 µg/mL) also significantly increased eNOS protein levels up to 1.8-fold. Furthermore, we found an increased human eNOS promotor activity (up to 1.9-fold) in response to red wine polyphenols (18 h, 100-600 µg/mL) as demonstrated by a human eNOS-luciferase reporter gene assay. We provide conclusive data showing for the first time that a RWPE increases eNOS expression and subsequent endothelial NO release. Increased active eNOS levels may antagonize the development of endothelial dysfunction and atherosclerosis supporting the view that red wine indeed may have long-term protective cardiovascular properties mediated by its polyphenols.

*Acknowledgements*: We thank Dr. Véronique Cheynier, INRA-UMR Sciences pour l'Oenologie, Montpellier for providing chemically characterized red wine polyphenol extract.

**References: 1.** Renaud, S. (1992) Lancet 339: 1523-1526. **2.** Li, H. et al. (2000) J Pathol. 190: 244-254.

## SL10 Selective estrogenic activity of *Vitex agnus-castus*

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Extracts of the fruits of Vitex *agnus-castus* (VAC) are commonly used for the treatment of premenstrual symptoms, corpus luteum insufficiency and menstrual cycle length disorders. In our screening programme for plant extracts with estrogen-like activity we also included VAC. In a receptor-binding assay performed with recombinant human ER the VAC extract BNO 1095 showed a preferential binding to ERβ over ERα, thus revealing the quality of a phyto-SERM.

The extract was fractionated at Sephadex LH20 as stationary phase with 75% (v/v) ethanol as mobile phase. Column dimensions were 500 cm x 5 cm, flow rate was adjusted with a pump to 1 mL/min. Aliquots of the effluent were tested for their binding strength to a cytosolic preparation from porcine uteri. Active fractions from this assay were collected and submitted for differentiation reasons to a second binding assay to human recombinant ERα or ERβ. The compound did not bind to the ERα but had clear affinity towards the ERβ.

Elucidation of the structure of the active compound was achieved by TLC, comparison of retention times in HPLC, UV-VIS-spectroscopy, $^{1}$H- and $^{13}$C-NMR. The physico-chemical data of the isolated compound and apigenin used as reference compound were identical.

Apigenin has been described to possess preferential binding activity to ERβ over ERα by Kuiper *et al.* but to our knowledge the compound was not yet described to occur in VAC, even if the occurrence of vitexin and isovitexin in the plant make the presence of the aglycone plausible.

**References:** Kuiper, GG. et al. (1997), Endocrinology, 138: 863-870.