

## **EFFECTS OF VITAMIN C SUPPLEMENTATION ON HUMAN AORTIC ENDOTHELIAL CELLS REDOX STATUS AND NITRIC OXIDE PRODUCTION**

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Because standard culture media for human aortic endothelial cells (HAEC) do not contain vitamin C, we hypothesized that HAEC may be under significant oxidative insult compared to the situation *in vivo*. To assess parameters of oxidative stress, intracellular vitamin C, glutathione (GSH), GSH/GSSG ratio as well as oxidant appearance and oxidative damage were measured in HAEC with or without vitamin C addition.

The effect of vitamin C on eNOS activity was determined since it has been previously shown to increase nitric oxide production.

Results showed that HAEC without vitamin C treatment were essentially scorbutic. Intracellular vitamin C levels rose to 529 pmol/mg protein 30 min after vitamin C addition. GSH levels were unchanged by vitamin C treatment but the GSH/GSSG ratio increased from  $61.28 \pm 3.54$  to  $105.66 \pm 15.92$ . Significantly lower ( $P < 0.05$ ) oxidant appearance and steady-state oxidative damage were also observed following vitamin C repletion. Vitamin C treatment increased eNOS activity by 600%. Thus, HAEC are scorbutic under normal culture conditions and exhibit higher oxidative stress than vitamin C repleted cells.

Vitamin C supplementation should be considered when using cultured cells, especially when experimental endpoints are related to cellular redox status and eNOS activity.