*S*ex-dependent properties of male and female human umbilical vein endothelial cells (HUVECs): focus on eNOS

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Atherosclerosis and cardiovascular diseases (CVDs) are classical examples of diseases where sex/gender differences have been described. A significant body of evidence suggests that CVDs are less prevalent in women than men until midlife, and the female advantage has been attributed to estrogens, which are lost with menopause. Since the earliest event in the onset of atherosclerosis and CVDs is endothelial dysfunction (ED) - a reduced release of nitric oxide (NO) coupled with an increase in Reactive Oxygen Species (ROS) in the vascular wall - many *in vitro* studies have been focused on endothelial cells (ECs). However, the sex of ECs has not been consistently reported in these studies, even when the effects of sex hormones were analyzed.

To better study the influence of estrogens on metabolic properties of human male and female umbilical vein ECs (HUVECs), we cultured HUVECs in a nominally hormone-free medium - consisting of a phenol-red free medium supplemented with charcoal-stripped serum (CS-FBS) - typically used to assess i*n vitro* hormone biological activities. We found that HUVECs of both sexes stopped to grow and to sprout in the absence of hormones, and neither 17-β2 estradiol nor dihydrotestosterone reverted these inhibitory effects. We concluded that HUVEC growth and sprouting critically require some serum components - other than sex hormones - that are lost in the CS-FBS. We focused our attention on fatty acids (FAs) because: 1) metabolic pathways are emerging as important regulators of angiogenesis; 2) FAs have been crucially involved in the regulation of EC proliferation; 3) CS-FBS is fully depleted from FAs. As a matter of fact, the inhibitory effects on MTT absorbance, cell number and 3-D sprouting observed in HUVECs cultured in CS-FBS containing media were reverted by adding back sodium acetate and palmitic acid. These data confirm a fundamental role for FAs in the regulation of EC growth and sprouting. However, no substantial differences were found between male and female EC behavior in these conditions.

To further investigate inborn sex differences in ECs, we focused on a relevant issue that is the role of eNOS and of its product NO. Endothelium-produced NO has important functions on ECs themselves, playing a key role not only in CVD onset and development, but also in angiogenesis, by stimulating EC proliferation, migration and differentiation. We found that female HUVECs constitutively expressed an higher amount of eNOS both at mRNA and protein level. Moreover, female HUVECs possess greater migratory and 3-D spheroid sprouting capabilities in comparison to male cells. The increased migratory and angiogenic capabilities observed in female HUVECs were counteracted by the pretreatment with the NO synthesis inhibitor L-NAME.
These preliminary results suggest that the constitutive higher expression of eNOS observed in female HUVECs might contribute to the protection against CVDs characteristic of the younger female population. We will carry out further studies on ECs from different sources and ages to determine if the increase in eNOS expression observed in female HUVECs is preserved during lifetime and in ECs obtained from different vascular bed.