

## Physical Inactivity-induced Endothelial Dysfunction: The Role Toll-like Receptor 4

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**Background:** A hallmark characteristic of physical inactivity is not only the rapid deterioration of cardiovascular function and whole body insulin sensitivity, but also the accumulation of free fatty acids (FFAs) which contribute to vascular dysfunction, specifically endothelial cell dysfunction as a consequence of a mismatch between the synthesis and degradation of nitric oxide (NO). Although a mechanism that may contribute to physical inactivity-induced endothelial dysfunction is unclear, the accumulation of the sphingolipid ceramide within endothelial cells may activate Toll-like receptor (TLR) 4, which finally leads to vascular dysfunction. Therefore, this study sought to investigate the role of TLR4 signaling in physical-inactivity (hindlimb unloading) induced endothelial dysfunction.

**Methods:** Adult female wild type (WT) mice were separated in three groups ; control (CON) (n=8), hindlimb unloading for 14 days (HU) (n=8), and hindlimb unloading with toll-like receptor 4 inhibitor, TAK-242 (4mg/kg) (HUT) (n=8). Following 14 days, using an *in vitro* preparation, endothelium-dependent and -independent vasodilation were assessed in arteries in response to three stimuli 1) flow-induced shear stress, 2) acetylcholine (ACh), and 3) sodium nitroprusside (SNP). Additionally, L-N<sup>G</sup>-monomethyl arginine citrate (L-NMMA) was utilized to identify the impact of blocking NO synthase on these responses. Endothelial NOS (eNOS), TLR4, NFκB protein expression were analyzed via Western blot analysis.

**Results:** After 14 days of physical inactivity, HU group showed that progressive physical inactivity-induced reductions in endothelial-dependent vasodilatory function were present in response to the intraluminal flow (CON:  $81 \pm 3$ ; HU:  $40 \pm 2\%$ ,  $P < 0.05$ ) and ACh (CON:  $93 \pm 4$ , HU:  $44 \pm 3\%$ ,  $P < 0.05$ ). However, TLR4 inhibition using TAK-242, significantly restored endothelial function following physical inactivity; intraluminal flow (HUT:  $73 \pm 2\%$ ,  $P < 0.05$ ) and ACh (HU:  $85 \pm 3\%$ ,  $P < 0.05$ ). All groups had no significant effect on endothelium-independent vasodilation (SNP). P-eNOS/eNOS protein expression was significantly decreased with physical inactivity but TAK-242 prevented HU induced eNOS reduction. In addition, HU significantly activated an inflammation marker, NFκB protein expression, but TAK-242 prevented physical inactivity-induced NFκB protein expression.

**Conclusion:** Overall, these findings suggest that physical inactivity induces endothelial dysfunction and endothelial TLR4 signaling may play a critical role in physical inactivity-associated endothelial dysfunction. Therefore, TLR4 signaling could be a novel therapeutic target to restore endothelial function and NO bioavailability.