exhibit increased expression of the proliferation marker, PCNA (0.162 fold change, p < 0.05). VSMCs from HT subjects change their phenotype in hypertension. The VSMC oxidative proteome analysis identified 130 significantly regulated proteins (including ABCA1, ABCA2, IL1RAP, CD36, ICAM1) were also increased in cells from HT. Pro-fibrotic and mitogenic phenotype of VSMCs was assessed by measuring protein expression after transfection with small interfering RNA. Protein expression was detected by western blotting. The inflammatory, and hypertensive (HT) subjects were studied (n = 5). Proteins were labelled with isobaric tandem mass tags and identified by liquid chromatography tandem mass spectrometry. The oxidative proteome was assessed using stable isotope-labelled iodoacetamide to target cysteine thiol. Nox5 silencing was performed by siRNA. Protein expression was detected by western blotting. The inflammatory, pro-fibrotic and mitogenic phenotype of VSMCs was assessed by measuring pro-inflammatory cytokines (IL-6, IL-8), pro-collagen I in the culture media.

Results: Proteomic analysis identified 207 proteins upregulated in HT subjects (fold change>1.5, p < 0.05). Gene ontology enrichment analysis of upregulated proteins in HT showed most proteins belong to extracellular space and plasma membrane compartments and were involved in extracellular matrix organization (ECM), immune response and cell proliferation. ECM proteins (COL1A1, COL10A1, FN1, FBLN1) were increased in cells from HT, suggesting a switch to a fibroblast-like phenotype in hypertension. Expression of proteins related to interferon and IL-1β pathways (IFIT1, IFIT2, IFIT3, MX1, MX2, ABCA1, ABCA2, IL1RAP, CD36, ICAM1) were also increased in cells from HT subjects. The VSMC oxidative proteome analysis identified 130 significantly regulated cysteine-containing peptides, 88 showed increased oxidation in HT (fold change>1.5, p < 0.05). Among the highest oxidized proteins in HT were ECM proteins, COL1A1, COL1A6, FN1 and FBLN2. VSMCs from HT subjects exhibited increased expression of the proliferation marker, PCNA (0.162 ± 0.3 vs NT:0.051 ± 0.04 relative fluorescence units, p < 0.05) and pro-collagen I (23.6 ± 2 vs NT:13.2 ± 0.3 ng/ml, p < 0.05). Production of pro-inflammatory cytokines IL-6 (501.8 ± 23.6 vs NT:121.7 ± 6.4 pg/ml) and IL-8 (373.6 ± 34.1 vs NT:262.5 ± 24.6 pg/ml, p < 0.05) were increased in HT. Nox5 silencing in VSMCs from HT subjects reduced PCNA expression (43%), pro-collagen I release (8%), baseline and LPS-induced IL-6 (30% baseline, 43% LPS-induced) and IL-8 (21% baseline, 23% LPS-induced) release (p < 0.05).

Conclusions: Our study provides new insights into the proteomic changes related to vascular phenotype in hypertension and demonstrated that Nox5 plays an important role in VSMC phenotypic switching associated with vascular injury and remodelling in hypertension.

**S-07-6 | NEUTROPHIL EXTRACELLULAR TRAPS AS MEDIATORS OF ENDOTHELIAL DAMAGE IN HYPERTENSION AND DIABETES**

Chloe Landry1,2, Fengxia Xiao1, Jean Francois Thibodeau2, Chet E Holtemann2, LiLu Xu1, Alex Gutsol1, Christopher RJ Kennedy1,2, Dylan Burger1,2 University of Ottawa, Canada, 2Ottawa Hospital Research Institute, Canada

Objective: Endothelial dysfunction (ED) plays a key role in the pathogenesis of hypertension and diabetes, yet its molecular determinants are poorly understood. Concomitant hypertensive and diabetes can synergistically increase damage to the vasculature, with increasing evidence suggesting that resulting immune dysregulation may contribute to endothelial injury. Neutrophil extracellular traps (NETs), networks of chromatin and protein-containing extracellular fibers released to fight infection, are generated from neutrophils following activation of the citrullinating enzyme peptidylarginine deiminase 4 (PAD4). PAD4 has been shown to be upregulated in atherosclerosis, vasculitis, and various inflammatory autoimmune diseases, leading to NET-induced endothelial injury. Whether NETs could contribute to hypertension- and diabetes-induced ED has not been established. The objective of the present study is to investigate the impact of hypertension and diabetes on PAD4 activity and the contribution of NETs to resulting vascular injury.

Design and Method: Promyelocytic HL-60 cells were differentiated to neutrophil-like cells and treated with angiotensin II (10−7 M) and/or high glucose (25 mM) for 24 hours, with or without subsequent NET-inducing PMA stimulation (500 nM). NETs were isolated from the culture medium by differential centrifugation and assessed by Western blot analysis. Our ongoing work will unravel the underlying mechanisms, and may ultimately lead to hormone-specific therapies aimed at stabilizing plaques and reducing the incidence of stroke for men and women.