# Editorial

# Beyond Impressions

## How Altered Shear Stress Connects Hypoxic Signaling to Endothelial Inflammation

Xueyi Li, Daniel S. Weintraub, David J.R. Fulton



Atherosclerotic lesions form preferentially in arterial regions characterized by slow and irregular patterns of blood flow such as those found on the inner curvature of bifurcation branch points. Because of this nonrandom distribution, extensive research has focused on the role of shear stress or the mechanical drag force exerted on the endothelial lining of blood vessels. Blood flow that follows a high shear stress, unimpeded laminar pattern encourages homeostatic mechanisms in the endothelium and protects against atherosclerosis. The transition from laminar to disturbed flow elicits changes in endothelial cell behavior that include increased inflammatory signaling through the activation of NF-κB, increased expression of leukocyte adhesion receptors, and the recruitment of immune cells. Focal areas exposed to detrimental shear stress, together with the synergistic effects of dyslipidemia, age, and hyperglycemia, initiate and promote the growth of atherosclerotic lesions. The mechanisms by which endothelial cells sense and respond to these changes in flow have been intensively studied, but gaps in our knowledge remain.

**See accompanying article on page 2087**

In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Feng et al[1] report on the ability of low shear stress to upregulate HIF-1α and glycolytic programming in endothelial cells. HIF-1α is a master regulator of the cellular response to hypoxia, and its expression is associated with changes in metabolism, inflammation, and angiogenesis.[2,3] The ability of low oxygen tensions to increase atherosclerosis in ApoE-null mice[4] and the genetic deletion of HIF-1α, selectively in endothelial cells[5] or in macrophages,[6] to protect against atherosclerosis collectively suggest that hypoxia plays a pathogenic role. Arterial blood carries abundant levels of oxygen, and, therefore, hypoxia has been hypothesized to occur deep within the core of large atherosclerotic lesions, and this is supported by the detection of low oxygen concentrations and HIF-1α expression within plaques in both animal models and humans.[7,8] An interesting observation by Feng et al[1] was that HIF-1α expression was selectively increased in the low-flow inner curvature of nonatherosclerotic porcine aorta and in cultured endothelial cells exposed to low shear stress in the presence of atmospheric oxygen (findings recently confirmed by others[9]). These data suggest that the upregulation of HIF-1α may also play a role in the initiation of atherosclerosis. How mechanical stress on the endothelium regulates HIF-1α expression was an important next question. Feng et al[1] found that low and oscillatory shear stress on endothelial cells increased the activation of NF-κB which drives expression of HIF-1α mRNA, as well as increased expression of Cezanne or OTUD7B, an editor of ubiquitin chains that preserves HIF-1α protein expression.[10] Although the ability of oscillatory shear to activate NF-κB is well described,[11] others have found that the ability of disturbed flow to induce HIF-1α expression is mediated instead by NOX4-derived reactive oxygen species in manner that is independent of NF-κB.[9] Important differences between these studies include the type of cell used (HUVEC [human umbilical vein endothelial cells] versus HAEC [human aortic endothelial cells][9]), the strategies to inhibit NF-κB (Rel siRNA, IκBα overexpression versus a NEMO binding domain peptide), and the approaches used to model disturbed flow (orbital shaking or an Ibidi parallel plate versus a cone and plane).

HIF-1α is a key transcription factor that orchestrates metabolic reprogramming in hypoxic cells toward glycolysis. Enhanced glycolysis can also occur in normoxic cells, a phenomena first described by Warburg.[12] Glycolysis not only supports enhanced rates of proliferation and migration but also has emerged as a powerful regulator of angiogenesis and inflammation.[13–15] Feng et al[1] and others found increased expression of glycolytic enzymes in normoxic endothelial cells exposed to low or disturbed flow in culture, as well as in partially ligated carotid arteries and atheroprone regions of porcine aorta.[9] HIF-1α and its family member, HIF-2α (EPAS1), were both upregulated by disturbed flow, but increased expression of glycolytic enzymes was dependent only on HIF-1α.[9] HIF-1α and NF-κB have a complicated interrelationship which is also observed in endothelial cells exposed to changes in flow. Hypoxia, HIF-1α, and increased expression of glycolytic enzymes are connected with increased inflammation,[16,17] and NF-κB can drive increased HIF-1α expression.[18] In endothelial cells exposed to disturbed flow, silencing both HIF-1α and select glycolytic enzymes decreases NF-κB activation and the expression of proinflammatory genes.[9] HIF-1α is not the only shear stress–sensitive transcription factor, and previous studies have identified *KLF2* as a gene that is strongly upregulated by laminar flow[19] and suppressed by disturbed flow.[9] In effects opposite to HIF-1α, KLF2 has been shown to repress inflammatory signaling[20] and glycolytic metabolism.[21] Whether KFL2 impacts disturbed flow–induced upregulation of HIF-1α is not yet known. This is an important question because KLF2 has been shown to potently inhibit HIF-1α expression and function.[22] In contrast, silencing HIF-1α in endothelial cells exposed to disturbed flow resulted in increased expression of

From the Vascular Biology Center, Medical College of Georgia, Augusta University.

Correspondence to David J.R. Fulton, PhD, Vascular Biology Center, Augusta University, Augusta, Georgia 30912. E-mail dfulton@augusta.edu

**(*Arterioscler Thromb Vasc Biol*. 2017;37:1987-1989. DOI: 10.1161/ATVBAHA.117.310149.)**



***Arterioscler Thromb Vasc Biol* is available at http://atvb.ahajournals.org**
**DOI: 10.1161/ATVBAHA.117.310149**

KLF2, suggesting that the mechanism by which disturbed flow decreases KLF2 expression is via increased HIF-1α.[9]

The posttranslational modification of proteins by the addition of ubiquitin, a small 8.5 kDa ubiquitous protein, to select lysine residues is an important regulator of protein function and cell signaling. Protein degradation is one of the best-known consequences of ubiquitin modification, but ubiquitin can also alter protein conformation and function and subcellular targeting.[23] In normoxic conditions, HIF-1α typically undergoes VHL-dependent ubiquitination and degradation.[24,25] An underappreciated aspect of ubiquitin modification is its reversibility, and proteins targeted for elimination can earn a reprieve through the actions of a group of enzymes known as deubiquitinating enzymes. The role of deubiquitinating enzymes in shear stress and atherosclerosis is poorly understood. Otud7b (Cezanne) is a deubiquitinating enzyme that belongs to A20 like ovarian tumor domain subfamily. Cezanne has been shown to specifically break ubiquitin chains linked to Lys11 but can also deubiquitinate Lys48 and Lys63, endowing it with potentially important roles in regulating protein stability and signaling.[26] Cezanne has emerged as an important regulator of both NF-κB signaling and HIF-1α expression.[10,27–29] In the study by Feng et al,[1] Cezanne was upregulated by low flow and its expression was necessary to stabilize NF-κB–induced HIF-1α protein expression. The inability of Cezanne to impact disturbed flow–induced upregulation of NF-κB is an apparent contradiction of previous findings.[28,30] The authors address this conundrum by suggesting that shear stress may activate NF-kB in a manner that is immune to Cezanne mediated deubiquitination.

In summary, Feng et al[1] have expanded our knowledge of the role of HIF-1α in the development of atherosclerosis. In specific they show that in addition to a role in regulating intraplaque angiogenesis in advanced lesions, HIF-1α also functions in the early stages of atherosclerosis to initiate lesion formation by promoting inflammatory signaling in arterial regions exposed to low or nonlaminar shear stress. Endothelial cells in culture and in regions of blood vessels exposed to low and turbulent flow have increased HIF-1α expression along with the upregulation of numerous glycolytic enzymes and increased inflammatory signaling through enhanced activation of NF-κB (Figure). Although these results are in excellent agreement with a recent publication,[9] important gaps in our knowledge remain including a better understanding of the shear stress–dependent signaling events leading to expression of HIF-1α. A role for NOX4 in shear stress–mediated induction and stabilization of HIF-1α as proposed by others[9] is complicated by numerous studies, showing that loss of NOX4 exacerbates atherosclerosis,[31] although there may be confounding temporal considerations. How shear stress impacts Cezanne expression is also ambiguous with some publications showing little to no effect compared with proinflammatory cytokine such as TNFα and upregulation by laminar flow.[27,32] The impact of KLF2 on HIF-1α expression and how Cezanne and other deubiquitinating enzymes affect shear stress–dependent changes in NF-κB await further clarification. VEGF is robustly upregulated by HIF-1α and has been shown to be proatherogenic,[33] but whether shear stress–dependent changes in VEGF have an important role is not known. The effect of low ambient oxygen concentrations on atherosclerosis is also complex; although 3-week exposure to hypoxia in ApoE-null mice[4] and chronic intermittent hypoxia increases lesion burden,[34] the long-term adaptation to hypoxia is protective in both mice and humans at altitude.[35]

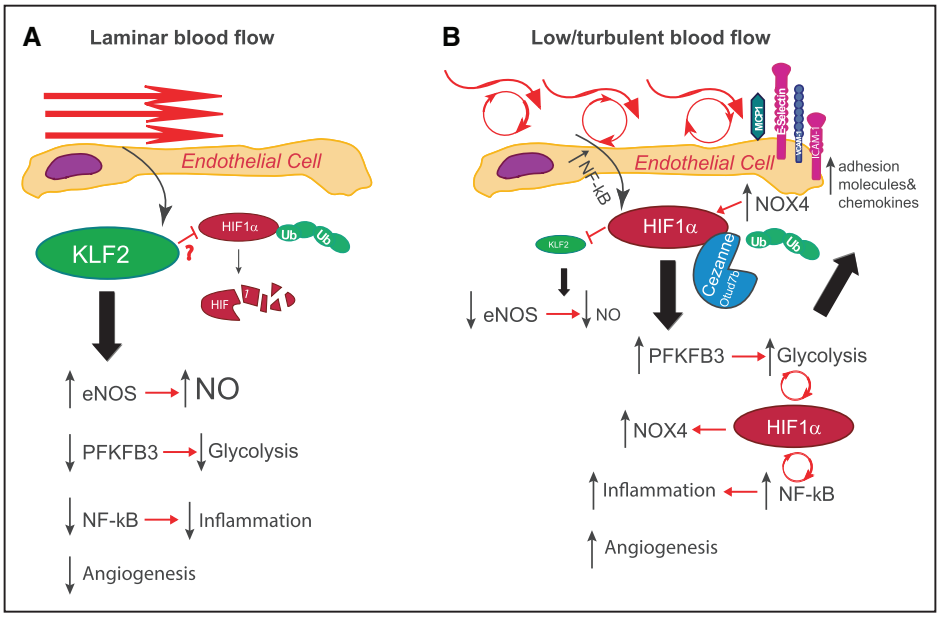


**Figure.** HIF-1α contributes to shear stress-dependent changes in metabolism and inflammation in endothelial cells. Mechanosensitive pathways in endothelial cells subject to (**A**) laminar flow or (**B**) disturbed flow. Laminar shear stress upregulates KLF2 which has been shown to inhibit HIF-1α by promoting its degradation and collectively these events lead to increased expression of homeostatic enzymes such as eNOS (endothelial nitric oxide synthase), inhibition of NF-κB and inflammation, decreased angiogenesis, and suppression of key glycolytic enzymes such as PFKFB3 (6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3). In contrast, turbulent flow and oscillatory shear stimulate NF-κB which induces HIF-1α resulting in the loss of KLF2. HIF-1α protein expression is stabilized by the upregulation of Cezanne which removes ubiquitin modifications and also by NOX4. HIF-1α drives increased glycolysis, inflammatory signaling via NF-κB and expression of adhesion molecules, and increased angiogenesis.

## Sources of Funding

This study was supported by grants from National Institutes of Health, 1R01HL124773-01A1, 1R01HL125926-01A1.

## Disclosures

None.

## References

1. Feng S, Bowden N, Fragiadaki M, et al. Mechanical activation of hypoxia-inducible factor 1α drives endothelial dysfunction at atheroprone sites. *Arterioscler Thromb Vasc Biol*. 2017;37:2087–2101. doi: 10.1161/ATVBAHA.117.309249.
2. Carmeliet P, Dor Y, Herbert JM, et al. Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. *Nature*. 1998;394:485–490. doi: 10.1038/28867.
3. Semenza GL. Hypoxia-inducible factors in physiology and medicine. *Cell*. 2012;148:399–408. doi: 10.1016/j.cell.2012.01.021.
4. Nakano D, Hayashi T, Tazawa N, Yamashita C, Inamoto S, Okuda N, Mori T, Sohmiya K, Kitaura Y, Okada Y, Matsumura Y. Chronic hypoxia accelerates the progression of atherosclerosis in apolipoprotein E-knockout mice. *Hypertens Res*. 2005;28:837–845. doi: 10.1291/hypres.28.837.
5. Akhtar S, Hartmann P, Karshovska E, Rinderknecht FA, Subramanian P, Gremse F, Grommes J, Jacobs M, Kiessling F, Weber C, Steffens S, Schober A. Endothelial hypoxia-inducible factor-1α promotes atherosclerosis and monocyte recruitment by upregulating microRNA-19a. *Hypertension*. 2015;66:1220–1226. doi: 10.1161/HYPERTENSIONAHA.115.05886.
6. Aarup A, Pedersen TX, Junker N, Christoffersen C, Bartels ED, Madsen M, Nielsen CH, Nielsen LB. Hypoxia-inducible factor-1alpha expression in macrophages promotes development of atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2016;36:1782–1790.
7. Sluimer JC, Gasc JM, van Wanroij JL, Kisters N, Groeneweg M, Sollewijn Gelpke MD, Cleutjens JP, van den Akker LH, Corvol P, Wouters BG, Daemen MJ, Bijnens AP. Hypoxia, hypoxia-inducible transcription factor, and macrophages in human atherosclerotic plaques are correlated with intraplaque angiogenesis. *J Am Coll Cardiol*. 2008;51:1258–1265. doi: 10.1016/j.jacc.2007.12.025.
8. Parathath S, Mick SL, Feig JE, Joaquin V, Grauer L, Habiel DM, Gassmann M, Gardner LB, Fisher EA. Hypoxia is present in murine atherosclerotic plaques and has multiple adverse effects on macrophage lipid metabolism. *Circ Res*. 2011;109:1141–1152. doi: 10.1161/CIRCRESAHA.111.246363.
9. Wu D, Huang RT, Hamanaka RB, Krause M, Oh MJ, Kuo CH, Nigdelioglu R, Meliton AY, Witt L, Dai G, Civelek M, Prabhakar NR, Fang Y, Mutlu GM. HIF-1alpha is required for disturbed flow-induced metabolic reprogramming in human and porcine vascular endothelium. *Elife*. 2017;6:e25217. doi: 10.7554/eLife.25217.
10. Bremm A, Moniz S, Mader J, Rocha S, Komander D. Cezanne (OTUD7B) regulates HIF-1α homeostasis in a proteasome-independent manner. *EMBO Rep*. 2014;15:1268–1277. doi: 10.15252/embr.201438850.
11. Tzima E, Irani-Tehrani M, Kiosses WB, Dejana E, Schultz DA, Engelhardt B, Cao G, DeLisser H, Schwartz MA. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. *Nature*. 2005;437:426–431. doi: 10.1038/nature03952.
12. Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nat Rev Cancer*. 2011;11:85–95. doi: 10.1038/nrc2981.
13. Cheng SC, Quintin J, Cramer RA, et al. mTOR- and HIF-1α-mediated aerobic glycolysis as metabolic basis for trained immunity. *Science*. 2014;345:1250684. doi: 10.1126/science.1250684.
14. Schoors S, De Bock K, Cantelmo AR, et al. Partial and transient reduction of glycolysis by PFKFB3 blockade reduces pathological angiogenesis. *Cell Metab*. 2014;19:37–48. doi: 10.1016/j.cmet.2013.11.008.
15. Xu Y, An X, Guo X, et al. Endothelial PFKFB3 plays a critical role in angiogenesis. *Arterioscler Thromb Vasc Biol*. 2014;34:1231–1239. doi: 10.1161/ATVBAHA.113.303041.
16. Koong AC, Chen EY, Giaccia AJ. Hypoxia causes the activation of nuclear factor kappa B through the phosphorylation of I kappa B alpha on tyrosine residues. *Cancer Res*. 1994;54:1425–1430.
17. Cramer T, Yamanishi Y, Clausen BE, Förster I, Pawlinski R, Mackman N, Haase VH, Jaenisch R, Corr M, Nizet V, Firestein GS, Gerber HP, Ferrara N, Johnson RS. HIF-1alpha is essential for myeloid cell-mediated inflammation. *Cell*. 2003;112:645–657.
18. Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel AS, Nizet V, Johnson RS, Haddad GG, Karin M. NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. *Nature*. 2008;453:807–811. doi: 10.1038/nature06905.
19. Dekker RJ, van Soest S, Fontijn RD, Salamanca S, de Groot PG, VanBavel E, Pannekoek H, Horrevoets AJ. Prolonged fluid shear stress induces a distinct set of endothelial cell genes, most specifically lung Krüppel-like factor (KLF2). *Blood*. 2002;100:1689–1698. doi: 10.1182/blood-2002-01-0046.
20. SenBanerjee S, Lin Z, Atkins GB, Greif DM, Rao RM, Kumar A, Feinberg MW, Chen Z, Simon DI, Luscinskas FW, Michel TM, Gimbrone MA Jr, García-Cardeña G, Jain MK. KLF2 Is a novel transcriptional regulator of endothelial proinflammatory activation. *J Exp Med*. 2004;199:1305–1315. doi: 10.1084/jem.20031132.
21. Doddaballapur A, Michalik KM, Manavski Y, Lucas T, Houtkooper RH, You X, Chen W, Zeiher AM, Potente M, Dimmeler S, Boon RA. Laminar shear stress inhibits endothelial cell metabolism via KLF2-mediated repression of PFKFB3. *Arterioscler Thromb Vasc Biol*. 2015;35:137–145. doi: 10.1161/ATVBAHA.114.304277.
22. Kawanami D, Mahabeleshwar GH, Lin Z, Atkins GB, Hamik A, Haldar SM, Maemura K, Lamanna JC, Jain MK. Kruppel-like factor 2 inhibits hypoxia-inducible factor 1alpha expression and function in the endothelium. *J Biol Chem*. 2009;284:20522–20530. doi: 10.1074/jbc.M109.025346.
23. Schwartz AL, Ciechanover A. Targeting proteins for destruction by the ubiquitin system: implications for human pathobiology. *Annu Rev Pharmacol Toxicol*. 2009;49:73–96. doi: 10.1146/annurev.pharmtox.051208.165340.
24. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, von Kriegsheim A, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW, Ratcliffe PJ. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. *Science*. 2001;292:468–472. doi: 10.1126/science.1059796.
25. Kamura T, Sato S, Iwai K, Czyzyk-Krzeska M, Conaway RC, Conaway JW. Activation of HIF1alpha ubiquitination by a reconstituted von Hippel-Lindau (VHL) tumor suppressor complex. *Proc Natl Acad Sci USA*. 2000;97:10430–10435.
26. Mevissen TET, Kulathu Y, Mulder MPC, Geurink PP, Maslen SL, Gersch M, Elliott PR, Burke JE, van Tol BDM, Akutsu M, Oualid FE, Kawasaki M, Freund SMV, Ovaa H, Komander D. Molecular basis of Lys11-polyubiquitin specificity in the deubiquitinase Cezanne. *Nature*. 2016;538:402–405. doi: 10.1038/nature19836.
27. Enesa K, Zakkar M, Chaudhury H, Luong le A, Rawlinson L, Mason JC, Haskard DO, Dean JL, Evans PC. NF-kappaB suppression by the deubiquitinating enzyme Cezanne: a novel negative feedback loop in pro-inflammatory signaling. *J Biol Chem*. 2008;283:7036–7045. doi: 10.1074/jbc.M708690200.
28. Luong le A, Fragiadaki M, Smith J, Boyle J, Lutz J, Dean JL, Harten S, Ashcroft M, Walmsley SR, Haskard DO, Maxwell PH, Walczak H, Pusey C, Evans PC. Cezanne regulates inflammatory responses to hypoxia in endothelial cells by targeting TRAF6 for deubiquitination. *Circ Res*. 2013;112:1583–1591. doi: 10.1161/CIRCRESAHA.111.300119.
29. Abe J, Berk BC. Cezanne paints inflammation by regulating ubiquitination. *Circ Res*. 2013;112:1526–1528. doi: 10.1161/CIRCRESAHA.113.301518.
30. Enesa K, Evans P. The biology of A20-like molecules. *Adv Exp Med Biol*. 2014;809:33–48.
31. Fulton DJ, Barman SA. Clarity on the isoform-specific roles of NADPH oxidases and NADPH oxidase-4 in atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2016;36:579–581. doi: 10.1161/ATVBAHA.116.307096.
32. Passerini AG, Polacek DC, Shi C, Francesco NM, Manduchi E, Grant GR, Pritchard WF, Powell S, Chang GY, Stoeckert CJ Jr, Davies PF. Coexisting proinflammatory and antioxidative endothelial transcription profiles in a disturbed flow region of the adult porcine aorta. *Proc Natl Acad Sci USA*. 2004;101:2482–2487.
33. Celletti FL, Waugh JM, Amabile PG, Brendolan A, Hilfiker PR, Dake MD. Vascular endothelial growth factor enhances atherosclerotic plaque progression. *Nat Med*. 2001;7:425–429. doi: 10.1038/86490.
34. Savransky V, Nanayakkara A, Li J, Bevans S, Smith PL, Rodriguez A, Polotsky VY. Chronic intermittent hypoxia induces atherosclerosis. *Am J Respir Crit Care Med*. 2007;175:1290–1297. doi: 10.1164/rccm.200612-1771OC.
35. Kang JG, Sung HJ, Amar MJ, Pryor M, Remaley AT, Allen MD, Noguchi AC, Springer DA, Kwon J, Chen J, Park JH, Wang PY, Hwang PM. Low ambient oxygen prevents atherosclerosis. *J Mol Med*. 2016;94:277–286. doi: 10.1007/s00109-016-1386-3.

KEY WORDS: Editorials ■ endothelial cells ■ hypoxia ■ inflammation ■ shear stress ■ ubiquitin

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION



# Beyond Impressions: How Altered Shear Stress Connects Hypoxic Signaling to Endothelial Inflammation

Xueyi Li, Daniel S. Weintraub and David J.R. Fulton

*Arterioscler Thromb Vasc Biol.* 2017;37:1987-1989
doi: 10.1161/ATVBAHA.117.310149
*Arteriosclerosis, Thrombosis, and Vascular Biology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1079-5642. Online ISSN: 1524-4636