

Effects of Reduced Cyclic Stretch on Vascular Smooth Muscle Cell Function of Pig Carotids Perfused *Ex Vivo*

Veronica Gambillara¹, Tyler Thacher¹, Paolo Silacci¹ and Nikos Stergiopoulos¹

BACKGROUND

With advancing age arteries stiffen, reducing arterial compliance and leading to the development of systolic hypertension and to a substantial increase in pulse pressure. An augmented pulse pressure can be a predictor of the development of hypertension, which has been linked to several cardiovascular diseases including atherosclerosis, and to pathologies such as diabetes and renal dysfunction. In this study, we tested the hypothesis that reduced wall compliance induces pulse-pressure-mediated changes in arterial wall metabolism and remodeling.

METHODS

Porcine carotid arteries were perfused for 24 h using an *ex vivo* arterial support system. Control arteries were exposed to a pulse shear stress (6 ± 3 dynes/cm²) combined with a pulse pressure of 80 ± 10 mm Hg, yielding a physiological cyclic stretch of 4–5%. A reduced compliance group was also studied, in which arteries were wrapped with an external band, thereby decreasing cyclic stretch to levels <1%.

RESULTS

The experimentally reduced compliance caused a decreased contraction capacity induced by norepinephrine (NE), and this was associated with lower levels of α -smooth muscle cell-actin (α -SMC-actin) and desmin protein expressions. Arteries that were exposed to a reduced cyclic stretch exhibited a higher level of matrix metalloproteinase-2 (MMP-2) expression activity as well as an increase in Ki67 expression, thereby suggesting that matrix degradation and cellular proliferation had been initiated. Furthermore, the expression of plasminogen activator inhibitor-1 (PAI-1) in stiffened arteries was lower than in the control arteries.

CONCLUSIONS

These findings underline the importance of cyclic stretch in the maintenance of a differentiated and fully functional phenotype of vascular SMCs, as well as in the regulation of migratory properties, proliferation, and matrix turnover.

Am J Hypertens 2008; **21**:425–431 © 2008 American Journal of Hypertension, Ltd.

Arterial stiffening is considered to be an accurate marker of the aging process, and is associated with several cardiovascular disease conditions such as hypertension and atherosclerosis, and with pathologies including diabetes and renal dysfunction.^{1,2} A consequence of arterial stiffening is an overall decrease in systemic compliance, augmenting vascular impedance and wave reflection, and leading to an increase in systolic and pulse pressures. Augmented pulse pressure is now recognized as a strong predictor of coronary heart disease.³ Another consequence of arterial stiffening is a decrease in arterial diameter pulsation over the heart cycle, or cyclic circumferential stretch.

Cyclic circumferential stretch is considered a potent biomechanical stimulus, a reduction in which can alter the smooth muscle cell (SMC) phenotype. Two proteins that are upregulated during SMC differentiation are α -SMC-actin,⁴ and desmin.⁵ α -SMC-actin is one of the most abundant proteins found in SMCs and is crucial to contractile function.⁶ Desmin is a crucial component in muscle-cell architecture

and is highly expressed in fully differentiated SMCs. Therefore, α -SMC-actin and desmin can be used as markers of SMC differentiation, providing insight into smooth muscle contractile characteristics with regard to phenotype.

A reduction in arterial compliance is also suspected to initiate certain cellular responses that are known to trigger the remodeling process, including cellular proliferation and migration rates,^{4,7,8} processes involved in atherosclerosis, and intimal wall thickening.^{9,10} Arterial remodeling is initiated by the breakdown of extracellular matrix, thereby increasing the migratory capabilities of cells moving from the media to the intima. Matrix metalloproteinases (MMPs) are a family of proteases capable of degrading extracellular matrix upon activation. MMP-2 has been implicated as a key player in arterial remodeling.¹¹ MMP activation, allowing for neo-intima development, may be regulated by the plasmin system,¹² namely plasminogen activator inhibitor-1 (PAI-1).¹³ Studying the regulation of MMP-2 and PAI-1 in response to altered wall compliance may provide an insight into certain aspects of arterial remodeling.

The effects of cyclic stretch on the regulation of endothelial cell signaling or SMC tone have been studied mostly in cells cultured either on stretch chambers or in elastic tubes, wherein cell–cell and matrix–cell interactions are missing. A few *in vivo* studies have been reported, wherein cyclic stretch has been

The first two authors contributed equally to this work.

¹Laboratory of Hemodynamics and Cardiovascular Technology, Swiss Federal Institute of Technology, Lausanne, Switzerland. Correspondence: Tyler Thacher (tyler.thacher@epfl.ch)

Received 10 September 2007; first decision 10 October 2007; accepted 4 December 2007; advance online publication 24 January 2008. doi:10.1038/ajh.2007.72

© 2008 American Journal of Hypertension, Ltd.

suppressed by wrapping a stiff band around a blood vessel. However, total suppression of cyclic stretch by a non-extensible cuff is not physiological and does not permit the study of reduced cyclic stretch because, with the exception of calcified arteries, some residual diameter pulsation always persists. We have therefore designed experiments in which arterial segments are perfused *ex vivo* under physiologically relevant pressure and flow conditions. Control arteries are subjected to either a physiological cyclic stretch (5%) or to a reduced cyclic stretch (1%). We addressed the short-term effects of reduced cyclic stretch on SMCs in their native tissue environment in which cell–cell and cell–matrix interactions are preserved. We focused our study on the effects of reduced cyclic stretch on SMC phenotype, contractile function, migration, proliferation, and induction of vascular remodeling.

METHODS

Arterial groups. Porcine left internal carotid arteries were obtained from the Bell slaughterhouse (Cheseaux, Switzerland) shortly after sacrifice of the animals, which were 6-month-old pigs weighing 120–150 kg. Adventitial tissue was removed and a 3.5 cm segment, 1 cm distal to the bifurcation was excised. After cleaning, the arterial segments were mounted onto the *ex vivo* arterial support system (see description later in the text). The segments were stretched longitudinally to 1.3 times the unstretched and unpressurized length. In order to simulate decreased compliance, a silicon cuff of 6.0 or 8.0 mm (depending upon the outer diameter), was placed around the arterial segment. The reduction in circumferential cyclic stretch obtained with the cuff was ~80%, as compared to the uncuffed arterial segment.

Arterial perfusion system. The *ex vivo* arterial perfusion system used in this study enables the perfusion of isolated arterial segments under precise control of perfusion pressure and flow. Details on *ex vivo* arterial support system have been given earlier.¹⁴ The arterial segments were perfused for 24 h with M199-EBS (Amersham) containing 5% fetal calf serum. The medium was constantly gas-infused with 5% CO₂ and 95% air. The perfusion flow to the arterial segments was adapted to create a pulsatile unidirectional shear stress with a mean value of 6 dyne/cm², amplitude of 3 dyne/cm² and frequency of 1 s. The mean perfusion pressure was set at 80 mm Hg, and pulse pressure amplitude was ±10 mm Hg. The resulting strain values were 4–5% for the uncuffed segment, which is in the physiological range of pulsatile stretch for the porcine carotid, and <1% for the cuffed segment, simulating a less compliant arterial segment.

It is worth noting that if shear stress is calculated on the basis of the diameter of a pressurized vessel under static conditions prior to the introduction of the pulsatile flow, slightly higher shear stresses will actually result in the cuffed vessel as compared to the non cuffed segment. In order to minimize this effect, the diameter should be measured first under pressurized, static flow conditions. Then, on the basis of this initial value, the flow rates should be set, and the shear stress calculated. Once

an acceptable flow and the desired cyclic stretch are achieved, the diameter is remeasured, taking its average value throughout the stretching cycle. This new value is then used for fine-tuning flow rates and shear stress. The flow is also rechecked throughout the 24-h perfusion, making fine adjustments, if necessary, in order to maintain the proper shear stress and cyclic stretching.

SMC analysis. The arterial rings were tested before and after the perfusion experiment to determine vasocontractility. They were then mounted in an organ chamber (EMKA Technology), equilibrated in Krebs solution at 37 °C, gassed with 95% O₂ and 5% CO₂. The resting tension was adjusted to 2 g. The arterial rings were pre-contracted with 60 mmol/l KCl until a constant maximum contraction was reached. Then norepinephrine (NE) and prostaglandin F₂-α (PG-F₂α) dose–response curves were obtained, with the NE and PG-F₂α concentrations varying between 10⁻⁸ mol/l and 10⁻⁵ mol/l. Endothelial functionality was a criterion for inclusion in our study, and was assessed using Bradykinin (Bk) (data not shown).

Protein extraction and analysis. Protein expression and gelatinolytic activity were assessed using immunoblot and zymography techniques, respectively, as described earlier.¹⁴ Protein was extracted from the samples with a Brij-35 lyses buffer (50 mmol/l Tris pH 7.5, 1 mol/l NaCl, 2 mol/l Urea, 0.1% Brij-35, and 1 protease inhibitor cocktail (Roche)). 20 μg of protein was subjected to electrophoresis, and then transferred to a nitrocellulose filter (Amersham). The filters were incubated with mouse anti-α-SMC-actin antibody¹⁵ (1:500, kind gift from the Gabbiani group in Geneva, Switzerland), mouse antidesmin antibody (1:500, Dako), rabbit anti-PAI-1 antibody (1:200, Santa Cruz Biotechnology), mouse antiMMP-2 antibody (1:500 Chemicon International), mouse anti-Glyceraldehyde-3-phosphate dehydrogenase antibody (1:500; VWR), followed by enhanced chemiluminescence–peroxidase-labeled anti-mouse or anti-rabbit antibodies (1:1000, Amersham). Protein expressions were normalized to the Glyceraldehyde-3-phosphate dehydrogenase protein expression. MMP-2 activity was determined using gel zymography and expressed as the ratio of the active form over the total MMP-2 (latent and active forms) for each sample.

Immunofluorescence and in situ zymography. At the end of the perfusion period, part of the arterial segment was rinsed with 0.9% NaCl, snap-frozen in OCT compound (Tissue-Tek) and stored at –80 °C for later analysis. Serial sections of 5 μm were cut, air-dried, and fixed in 100% acetone for 5 min at –20 °C for carrying out α-SMC-actin, desmin, Ki67, and PAI-1 staining. The sections were incubated with 0.1% Triton X-100 in phosphate-buffered saline for 10 min, then incubated for 60 min with α-SMC-actin (1:20, kind gift from the Gabbiani group in Geneva, Switzerland), desmin (1:50, Dako), Ki67 (1:40, Chemicon), or with rabbit anti-PAI-1 antibody (1:20, Santa Cruz Biotechnology) in 10% normal goat serum in phosphate-buffered saline. The sections were then incubated with a rabbit anti-mouse immunoglobulin G rabbit fluorescein-conjugate (1:100, Amersham) or a goat anti-mouse

immunoglobulin G rhodamine-conjugate (1:250, Amersham) as secondary antibody for 45 min. All steps were performed at room temperature. The sections were examined using a Zeiss Axiovert 135 microscope.

For detection of apoptosis, an *in situ* cell death detection kit was used (Roche). Cryopreserved tissue sections were fixed and permeated. 50 μ l of a TdT-mediated dUTP nick end labeling reaction mixture was added on the sample and stored for 60 min at 37°C in the dark. After being washed with phosphate-buffered saline, the samples were analyzed under a fluorescence microscope. Negative and positive samples have been used in order to validate the experiment. For the purpose of quantifying the apoptotic cells, we normalized the positive TdT-mediated dUTP nick end labeling stained cells to the total number of cells counted through nucleus detection of 4,6-diamidino-2-phenylindole in four different arteries in each group.

For *in situ* zymography, vessel sections were incubated at 37°C for 5 h with a fluorogenic gelatin substrate (DQ gelatin; Molecular Probes) at a concentration of 25 μ g/ml in a zymography buffer. For evaluating the hypothesis that increases in fluorescence were caused by enhanced MMP activity, for some of the arterial sections an inhibitor of such enzymes, 1-10-phenanthroline, was also added to the reaction buffer at a final concentration of 50 μ mol/l. Proteolytic activity was detected through green fluorescence (530 nm).

Statistics. The data are reported as mean values \pm s.d. Student's *t*-tests were performed to assess significant differences. A value of $P < 0.05$ was considered significant.

RESULTS

SMC contraction capacity in response to cyclic stretch

The results were arrived at by first normalizing the percentage change in contraction after addition of of NE and PG-F2 α (in concentrations ranging between 10⁻⁸ and 10⁻⁴ mol/l) to the contraction obtained with 60 mmol/l of KCl. This protocol was performed for the freshly harvested arteries, the normal group, and the reduced-stretch group. The contractile data from each normal and reduced-stretch experiment was then subtracted from the contractile data obtained from its corresponding freshly harvested segment. Therefore the dose-response curves for NE and PG-F2 α represent the average absolute increase in contractile response caused by the perfusion conditions.

NE-derived contraction capacity rose by 60% in arteries submitted to a normal cyclic stretch when compared with arteries exposed to a reduced cyclic stretch (Figure 1a, $P < 0.01$), but this rise in contraction capacity manifested itself only at higher concentrations of NE.

PG-F2 α , which followed the same trends as NE at lower concentrations but with a much lower overall contractile response, exhibited insignificant results (Figure 1b, $P < 0.1$). The largest difference in PG-F2 α -mediated contraction between our reduced and normal stretch cases, occurred at a dosage of 1.E-7 mol/l (not significant). The largest absolute values of con-

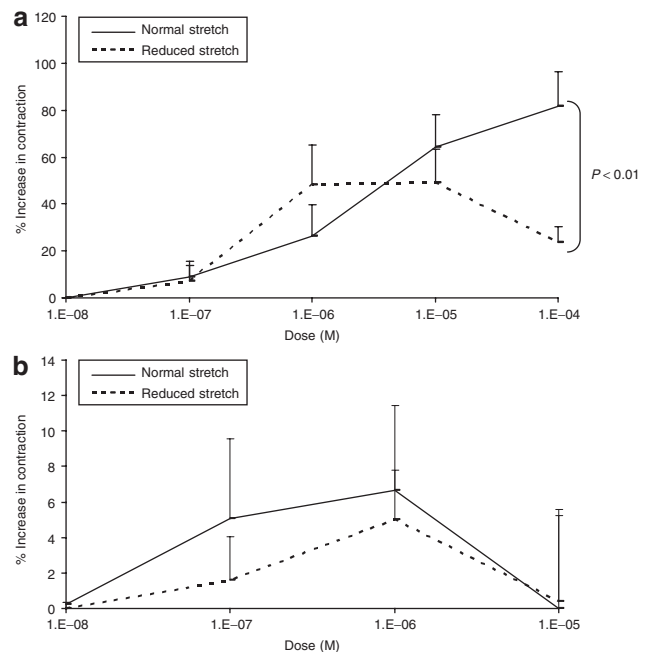


Figure 1 | Norepinephrine and prostaglandin F2- α dose-response curves. (a) Norepinephrine dose-dependent constriction capacity in porcine carotid artery segments after 1 day of *ex vivo* perfusion with a normal cyclic stretch (4–5%) and a reduced stretch (<1%). The data are represented as mean values \pm s.d., $n = 6$. (b) Prostaglandin F2- α dose-dependent constriction capacity in porcine carotid artery segments immediately after harvesting and after 1 day of *ex vivo* perfusion with a normal cyclic stretch (4–5%) and a reduced stretch (<1%). The data are expressed as mean values \pm s.d., $n = 3$.

traction occurred at 1.E-6 (not significant). At the final point, 1.E-5, both values approach zero, signifying that the average differences in contractile response in our experiments do not differ from those of the control segment.

Influence of cyclic stretch on SMC phenotype

In order to investigate the effect of cyclic stretch on SMC phenotype, we assessed the protein expression of α -SMC-actin and desmin, both markers of SMC differentiation. A significant difference in SMC phenotype was observed between the arteries exposed to physiological stretch and those exposed to reduced cyclic stretch. Reducing cyclic stretch causes a decrease of 30% in the protein expression α -SMC-actin (Figure 2a, $P < 0.05$) and 85% in that of desmin (Figure 2a, $P < 0.02$). These results were further supported by immunofluorescence of arterial sections. In arteries submitted to a normal stretch (Figure 2b, left), desmin expression could be easily detected in the media, whereas in sections with reduced compliance very little desmin expression was found (Figure 2b, right).

Effect of reduced cyclic stretch on MMPs expression and activation

In arteries exposed for 24 h to reduced cyclic stretch, MMP-2 expression was 50% higher than in those exposed to normal cyclic stretch (Figure 3a, $P < 0.01$). Zymography assays also revealed a higher activation of MMP-2 in arteries exposed to a reduced stretch (Figure 3b, $P < 0.05$).

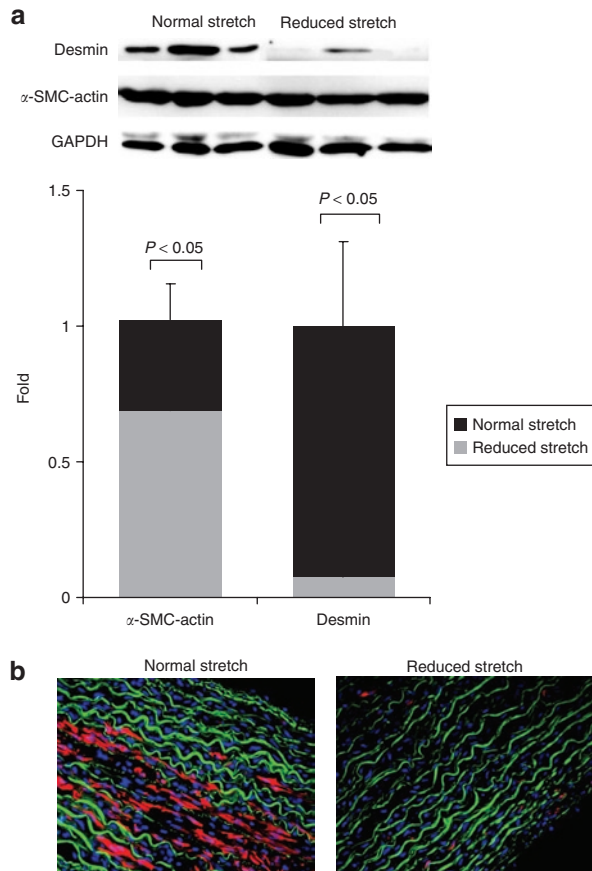


Figure 2 | Desmin protein expression. (a) Representative immunoblot showing α -smooth muscle cell-actin (α -SMC-actin) and desmin protein bands in arteries exposed to normal and reduced cyclic stretch. The data are expressed as mean values \pm s.d., $n = 6$. (b) Desmin was localized by immunostaining on cross-sections of arteries exposed to normal and reduced cyclic stretch. Desmin is seen in red, elastin in green, and nuclei in blue. All images were taken at identical contrast settings and luminescence levels at $\times 20$ original magnification. $n = 4$. GAPDH, glyseraldehyde-3-phosphate dehydrogenase.

In order to analyze the distribution of gelatinolytic activity throughout the arterial wall, we performed an *in situ* zymography. The increase in MMP expression that was observed in arteries exposed to reduced cyclic stretch correlated with the increase in gelatinolytic activity in the arterial wall (Figure 3c, A). This gelatinolytic activity was distributed mainly in the tunica media, and was present in both groups. Localization of gelatinolytic activity was reduced in the presence of the inhibitor 1-10-phenanthroline, thereby establishing the specificity of the assay (Figure 3c, B).

Effect of cyclic stretch on PAI-1 protein expression and localization

The expression level of PAI-1 was higher in arteries exposed to normal cyclic stretching. Cuffed arteries presented a 20% decrease in PAI-1 expression after 1 day of perfusion (Figure 4a, $P < 0.05$).

Immunostaining analysis allowed localization of PAI-1 expression, mainly in the media, in arteries exposed to normal

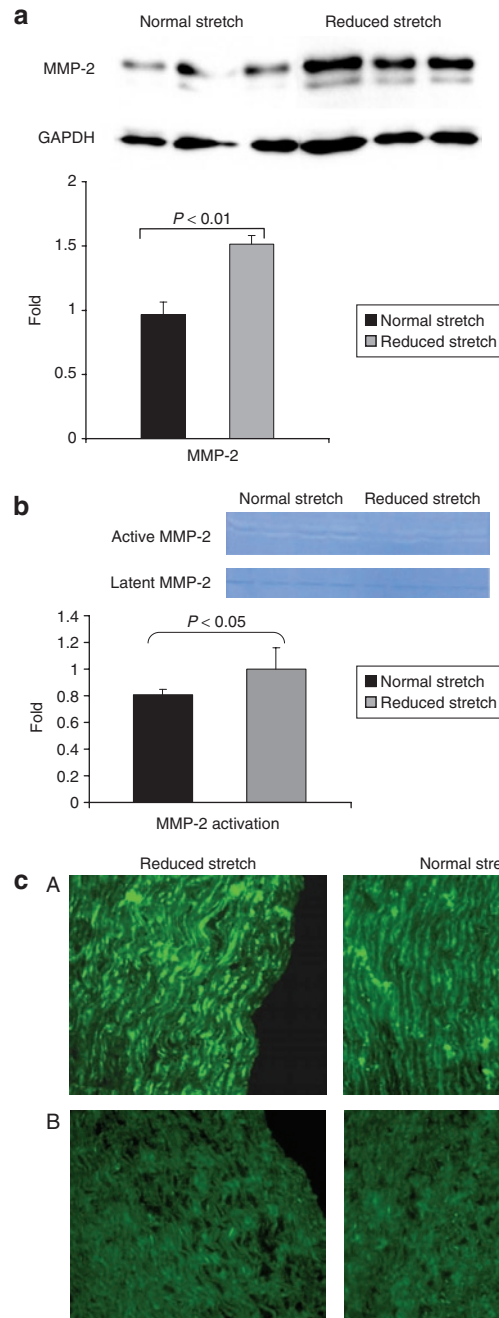


Figure 3 | Matrix metalloproteinase (MMP) expression. (a) The effect of cyclic stretch on MMP-2 protein expression, visualized by immunoblotting. MMP-2 protein expression was normalized to total glyseraldehyde-3-phosphate dehydrogenase (GAPDH) expression. The data are expressed as mean values \pm s.d., $n = 6$. (b) The effect of MMP-2 activity evaluated by zymography. The data are expressed as mean values \pm s.d., $n = 4$. (c) A, The effect of cyclic stretch on total MMP activation, as assessed by gelatinolytic activity; B: proof of specificity of the assay using the inhibitor 1-10-phenanthroline. All images were taken at identical contrast settings and luminescence levels at $\times 20$ original magnification. $n = 4$.

cyclic stretch (Figure 4b, left). By contrast, for arteries exposed to a reduced cyclic stretch, PAI-1 protein was downregulated and expressed in the media and endothelium (Figure 4b, right).

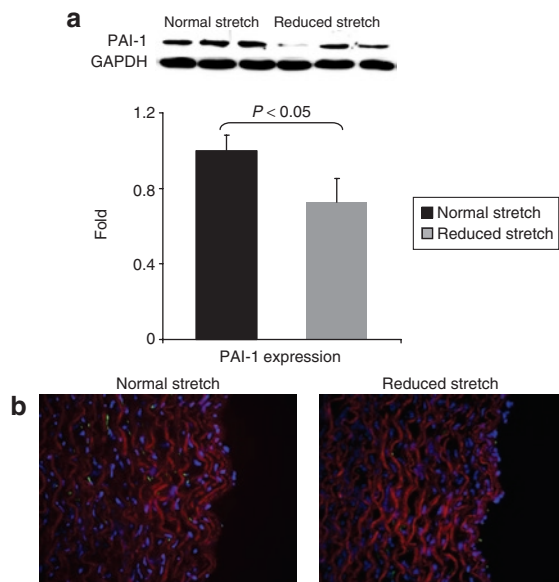


Figure 4 | Plasminogen activator inhibitor-1 (PAI-1) expression. **(a)** Representative immunoblot showing PAI-1 protein bands in arteries exposed to normal and reduced cyclic stretch. The data are expressed as mean values \pm s.d., $n = 6$. **(b)** PAI-1 was visualized by immunostaining on cross-sections of arteries exposed to normal and reduced cyclic stretch. PAI-1 is visualized in green, elastin in red, and nuclei in blue. All images were taken at identical contrast settings and luminescence levels at $\times 20$ original magnification. $n = 4$. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

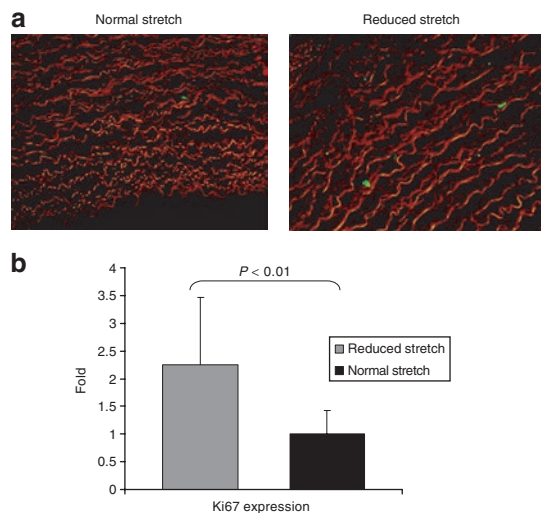


Figure 5 | Proliferation localization and expression. **(a)** Proliferation was visualized by staining arterial sections exposed to normal and reduced cyclic stretch with Ki67. Cells expressing Ki67 are seen in green and elastin in red. Images were taken at identical contrast settings and luminescence levels at $\times 20$ original magnification. $n = 4$. **(b)** The percentage of Ki67-positive cells per arterial section was calculated by dividing the number of positively stained cells (colocalized with a nucleus) by the total number of cells ($n = 4$).

Effect of cyclic stretch on proliferation

In order to investigate the effect of reduced cyclic stretch on cellular proliferation, we stained arteries exposed to normal and reduced cyclic stretch with Ki67. We noticed that proliferating cells were localized predominantly in the media (**Figure 5a**).

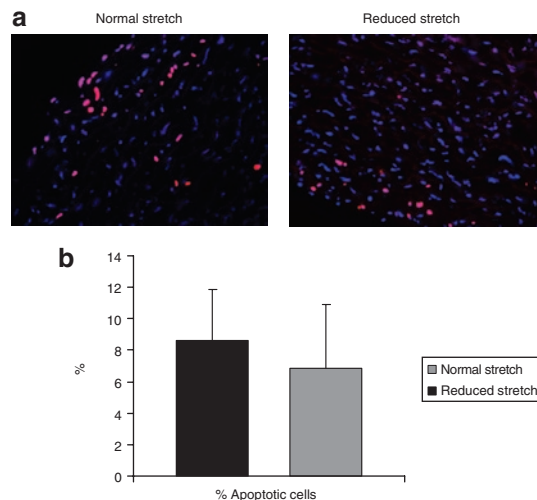


Figure 6 | Apoptosis localization and expression. **(a)** Apoptosis was visualized using a TdT-mediated dUTP nick end labeling reaction kit on cross-sections of arteries exposed for 1 day to normal and reduced cyclic stretch. Apoptotic cells are visualized in red and nuclei in blue. All images were taken at identical contrast settings and luminescence levels at $\times 20$ original magnification. $n = 6$. **(b)** The percentage of apoptotic cells in arterial sections was calculated by dividing the number of positively stained cells by the total number of cells ($n = 6$).

We observed a significant 2.2-fold increase in cellular proliferation in the reduced stretch group when compared with the normal stretch group (**Figure 5b**).

Effect of cyclic stretch on cell apoptosis

There was no statistical difference in apoptosis between the two groups of arterial segments (**Figure 6b**). Arteries exposed to normal cyclic stretch showed $9 \pm 3\%$ cell death, whereas arteries with artificially reduced cyclic stretch showed $7 \pm 3\%$ cell death, thereby suggesting that reducing the compliance does not induce higher apoptosis rates. We noticed that positive-stained cells were present mainly in the regions closer to the intima in both the groups (**Figure 6a**).

DISCUSSION

Effects of cyclic stretch on SMC phenotype and contractile function: Arterial stiffening decreases wall compliance and this, in turn, reduces cyclic stretch. Cyclic stretch is known to be a potent mechanical stimulus to endothelial cells and vascular SMCs. In order to study the effects of reduced cyclic stretch, we cuffed carotid arteries perfused under pulsatile pressure *ex vivo*. The experimentally reduced wall compliance induces changes in cellular responses and matrix turnover. Perfusion for 1 day with a reduced cyclic stretch leads to drastic decrease in contractile capacity when mediated by NE, of the order of 60% of arteries perfused with a normal cyclic stretch. PG-F2 α , which also induces a contractile response, although to a much smaller extent than NE, followed a similar trend to NE, yet yielded insignificant results. Contractile phenotype markers such as α -SMC-actin and desmin have been earlier shown to decrease in arteries submitted to a reduced cyclic stretch. In cuffed vessels, α -SMC-actin, the most abundant protein in the cytoskeleton, diminished by 30%, and the desmin expression

was almost completely removed. This suggests that reduced cyclic stretch leads to changes in SMC phenotype, apparently from contractile to synthetic. These results confirm earlier findings that cyclic stretch regulates the expression of some markers of SMC differentiation.^{4,8} In our experiments, arteries perfused under reduced cyclic stretch have shown a decreased contraction in response to NE when normalized to the maximal contraction induced by 60 mmol/l of KCl. Given the fact that the maximum contraction induced by KCl did not differ between our two groups, we hypothesize that the decrease in contractile response to NE in cuffed arteries is linked to a change in phenotype.^{16,17} Our hypothesis seems to be in accordance with the findings of Li *et al.*, who demonstrated that human spindle-shaped clones exhibit a higher expression of contractile phenotype markers and a stronger response to NE stimulation than do the epithelioid SMCs.¹⁶ The conclusions from the PG-F2 α data are more ambiguous, but they do suggest that reducing the cyclic stretch may also affect certain receptors responsible for SMC contraction.

Cyclic stretch in relation to vascular wall remodeling, migration, proliferation and apoptosis: Previously reported *in vitro* effects of mechanical strain on SMCs include increased cell proliferation and deposition of a matrix necessary for the organization and development of arteries, and later for vascular remodeling during atherosclerosis and hypertension.^{8,18} There is increasing evidence that changes in cyclic stretch lead to vascular wall remodeling, a process that involves degradation of the extracellular matrix through stimulation of MMPs.^{19–21} We observed an increase in MMP-2 expression in arteries submitted to a reduced cyclic stretch when compared with arteries submitted to a normal cyclic stretch. The activation of MMP-2 was also higher in the cuffed arteries. These results suggest that, in arteries with artificially reduced cyclic stretch, a vascular remodeling process is triggered, and this may lead to structural changes similar to those observed in *in vivo* studies.^{22–24} *In situ* zymography was used for assessing global gelatinase activity and for evaluating overall matrix turnover. When compared against data from the normal stretch group, these results confirm that under reduced cyclic stretch the matrix turnover cycle is being augmented.

Plasmin is a potent activator of most MMPs and is regulated by PAI-1. Downregulation of PAI-1 suggests an increased activation of MMPs.²⁵ Several groups of researchers have reported the involvement of MMPs and the fibrinolytic (plasmin/plasminogen) system as key factors in cell migration and tissue remodeling.^{13,26} We have shown a decrease in PAI-1 in arteries perfused with reduced cyclic stretch. Fluorescence data revealed that PAI-1 was predominantly localized at the endothelium, whereas, in arteries perfused with normal cyclic stretch, PAI-1 was primarily expressed by SMCs. Therefore a reduced circumferential cyclic stretch induces a simultaneous decrease in PAI-1 expression and increase in MMP-2 activity. These effects, together with cell migration and proliferation, are suspected to contribute to the increased intimal thickening.^{18,27} In addition, as discussed earlier, the cells in arteries with reduced cyclic stretch dedifferentiated into a

more synthetic phenotype known to have higher proliferation rates and mobile capacities.^{28,29}

In our study, we found no difference in apoptosis rates between the normal and reduced-stretch groups. This seems to partly contrast with the findings of Courtman *et al.*, who observed no changes in proliferation but marked an increase in apoptosis of SMCs in the media of infrarenal rabbit aortas which had been cuffed to reduce cyclic stretch. Courtman *et al.* also observed significant atrophy and necrosis. The differences may be attributable to differences in design and duration of the two studies. Courtman *et al.* performed long-term *in vivo* experiments and analyzed the consequences of the stiff external banding on vessel wall integrity after 6 weeks. Furthermore, their band was quite tight, reducing the diameter by 25% and totally unloading arterial media from circumferential stresses.³⁰ Our study diminished but did not totally abolish cyclic stretch, leaving a residual pulsation of the order of 1%, which is closer to the physiological pulsation in aged or stiffened conduit arteries. Also, our study describes changes in SMC phenotype and migration potential after only 1 day of *ex vivo* perfusion.

In summary, the current study demonstrates that a decrease in cyclic stretch leads to changes in SMC phenotypes, and to a concurrent decrease in NE-induced contraction capacity. Furthermore, the increase in MMP-2 is associated with the decrease in PAI-1 as well as with the increase in Ki67, thereby suggesting that the decrease in cyclic stretch may promote wall remodeling, SMC proliferation, and migration. Because reduction in cyclic stretch is often the result of a stiffened arterial tree, this work gives insights into possible consequences of arterial stiffening on large-vessel wall physio-pathology.

Acknowledgments: We would like to offer special thanks to Dominique Seppey and Chantal van den Broek for their help with this work.

Disclosure: The authors declared no conflict of interest.

- Zieman SJ, Melenovsky V, Kass DA. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol* 2005; 25:932–943.
- Lehoux S, Tedgui A. Signal transduction of mechanical stresses in the vascular wall. *Hypertension* 1998; 32:338–345.
- Benetos A, Safar M, Rudnichi A, Smulyan H, Richard JL, Ducimetière P, Guize L. Pulse pressure: a predictor of long-term cardiovascular mortality in a French male population. *Hypertension* 1997; 30:1410–1415.
- Reusch P, Wagdy H, Reusch R, Wilson E, Ives HE. Mechanical strain increases smooth muscle and decreases nonmuscle myosin expression in rat vascular smooth muscle cells. *Circ Res* 1996; 79:1046–1053.
- Watson PA, Hannan R, Carl LL, Giger KE. Desmin gene expression in cardiac myocytes is responsive to contractile activity and stretch. *Am J Physiol* 1996; 270:C1228–C1235.
- Saga H, Kimura K, Hayashi K, Gotow T, Uchiyama Y, Momiyama T, Tadokoro S, Kawashima N, Jimbou A, Sobue K. Phenotype-dependent expression of alpha-smooth muscle actin in visceral smooth muscle cells. *Exp Cell Res* 1999; 247:279–292.
- Langille BL. Arterial remodeling: relation to hemodynamics. *Can J Physiol Pharmacol* 1996; 74:834–841.
- Birukov KG, Shirinsky VP, Stepanova OV, Tkachuk VA, Hahn AW, Resink TJ, Smirnov VN. Stretch affects phenotype and proliferation of vascular smooth muscle cells. *Mol Cell Biochem* 1995; 144:131–139.
- Thyberg J, Hedin U, Sjolund M, Palmberg L, Bottger BA. Regulation of differentiated properties and proliferation of arterial smooth muscle cells. *Arteriosclerosis* 1990; 10:966–990.
- Liu SQ, Goldman J. Role of blood shear stress in the regulation of vascular smooth muscle cell migration. *IEEE Trans Biomed Eng* 2001; 48:474–483.

11. Abbruzzese TA, Guzman RJ, Martin RL, Yee C, Zarins CK, Dalman RL. Matrix metalloproteinase inhibition limits arterial enlargements in a rodent arteriovenous fistula model. *Surgery* 1998; 124:328–334; discussion 334–325.
12. Dewyer NA, Sood V, Lynch EM, Luke CE, Upchurch GR Jr, Wakefield TW, Kunkel S, Henke PK. Plasmin inhibition increases MMP-9 activity and decreases vein wall stiffness during venous thrombosis resolution. *J Surg Res* 2007; 142:357–363.
13. Peng L, Bhatia N, Parker AC, Zhu Y, Fay WP. Endogenous vitronectin and plasminogen activator inhibitor-1 promote neointima formation in murine carotid arteries. *Arterioscler Thromb Vasc Biol* 2002; 22:934–939.
14. Gambillara V, Montorzi G, Haziza-Pigeon C, Stergiopulos N, Silacci P. Arterial wall response to *ex vivo* exposure to oscillatory shear stress. *J Vasc Res* 2005; 42:535–544.
15. Skalli O, Ropraz P, Trzeciak A, Benzouana G, Gillissen D, Gabbiani G. A monoclonal antibody against alpha-smooth muscle actin: a new probe for smooth muscle differentiation. *J Cell Biol* 1986; 103:2787–2796.
16. Chien S, Li S, Shyy YJ. Effects of mechanical forces on signal transduction and gene expression in endothelial cells. *Hypertension* 1998; 31:162–169.
17. Sartore S, Franch R, Roelofs M, Chiavegato A. Molecular and cellular phenotypes and their regulation in smooth muscle. *Rev Physiol Biochem Pharmacol* 1999; 134:235–320.
18. Bendeck MP, Zempo N, Clowes AW, Galardy RE, Reidy MA. Smooth muscle cell migration and matrix metalloproteinase expression after arterial injury in the rat. *Circ Res* 1994; 75:539–545.
19. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. *Circ Res* 2002; 90:251–262.
20. Chesler NC, Ku DN, Galis ZS. Transmural pressure induces matrix-degrading activity in porcine arteries *ex vivo*. *Am J Physiol* 1999; 277:H2002–H2009.
21. Lehoux S, Lemaire CA, Esposito B, Lijnen HR, Tedgui A. Pressure-induced matrix metalloproteinase-9 contributes to early hypertensive remodeling. *Circulation* 2004; 109:1041–1047.
22. Benetos A, Laurent S, Hoeks AP, Boutouyrie PH, Safar ME. Arterial alterations with aging and high blood pressure. A noninvasive study of carotid and femoral arteries. *Arterioscler Thromb* 1993; 13:90–97.
23. Fath SW, Burkhart HM, Miller SC, Dalsing MC, Unthank JL. Wall remodeling after wall shear rate normalization in rat mesenteric arterial collaterals. *J Vasc Res* 1998; 35:257–264.
24. Galis ZS, Johnson C, Godin D, Magid R, Shipley JM, Senior RM, Ivan E. Targeted disruption of the matrix metalloproteinase-9 gene impairs smooth muscle cell migration and geometrical arterial remodeling. *Circ Res* 2002; 91:852–859.
25. Lijnen HR. Plasmin and matrix metalloproteinases in vascular remodeling. *Thromb Haemost* 2001; 86:324–333.
26. Redmond EM, Cullen JP, Cahill PA, Sitzmann JV, Stefansson S, Lawrence DA, Okada SS. Endothelial cells inhibit flow-induced smooth muscle cell migration: role of plasminogen activator inhibitor-1. *Circulation* 2001; 103:597–603.
27. Glagov S. Intimal hyperplasia, vascular modeling, and the restenosis problem. *Circulation* 1994; 89:2888–2891.
28. Bochaton-Piallat ML, Gabbiani G, Pepper MS. Plasminogen activator expression in rat arterial smooth muscle cells depends on their phenotype and is modulated by cytokines. *Circ Res* 1998; 82:1086–1093.
29. Hao H, Gabbiani G, Bochaton-Piallat ML. Arterial smooth muscle cell heterogeneity: implications for atherosclerosis and restenosis development. *Arterioscler Thromb Vasc Biol* 2003; 23:1510–1520.
30. Courtman DW, Cho A, Langille L, Wilson GJ. Eliminating arterial pulsatile strain by external banding induces medial but not neointimal atrophy and apoptosis in the rabbit. *Am J Pathol* 1998; 153:1723–1729.