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Artery Remodeling under Axial Twist in Three Days Organ Culture

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Abstract

Arteries often endure axial twist due to body movement and surgical procedures, but how arteries remodel under axial twist remains unclear. The objective of this study was to investigate early stage arterial wall remodeling under axial twist. Porcine carotid arteries were twisted axially and maintained for three days in *ex vivo* organ culture systems while the pressure and flow remained the same as untwisted controls. Cell proliferation, internal elastic lamina (IEL) fenestrae shape and size, endothelial cell (EC) morphology and orientation, as well as the expression of matrix metalloproteinases (MMPs), MMP-2 and MMP-9, and tissue inhibitor of metalloproteinase-2 (TIMP-2) were quantified using immunohistochemistry staining and immunoblotting. Our results demonstrated that cell proliferation in both the intima and media were significantly higher in the twisted arteries compared to the controls. The cell proliferation in the intima increased from $1.33 \pm 0.21\%$ to $7.63 \pm 1.89\%$, and in the media from $1.93 \pm 0.84\%$ to $8.27 \pm 2.92\%$ ($p < 0.05$). IEL fenestrae total area decreased from $26.07 \pm 2.13\%$ to $14.74 \pm 0.61\%$ and average size decreased from $169.03 \pm 18.85 \mu\text{m}^2$ to $80.14 \pm 1.96 \mu\text{m}^2$ ($p < 0.01$), but aspect ratio increased in the twisted group from 2.39 ± 0.15 to 2.83 ± 0.29 ($p < 0.05$). MMP-2 expression significantly increased ($p < 0.05$) while MMP-9 and TIMP-2 showed no significant difference in the twist group. The ECs in the twisted arteries were significantly elongated compared to the controls after three days. The angle between the major axis of the ECs and blood flow direction under twist was 7.46 ± 2.44 degrees after 3 days organ culture, a decrease from the initial 15.58 ± 1.29 degrees. These results demonstrate that axial twist can stimulate artery remodeling. These findings complement our understanding of arterial wall remodeling under mechanical stress resulting from pressure and flow variations.

Keywords

Axial Twist; Wall Remodeling; Matrix metalloproteinase; Internal elastic lamina; Endothelial cell morphology; Cell proliferation; *Ex vivo*; Artery; Porcine

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INTRODUCTION

Arteries are often subjected to axial twist along their longitudinal axes due to body movement and surgical procedures.^{3, 20, 28} Rotations of the head often induce considerable torsion in the common carotid and internal carotid arteries.⁴⁶ Vascular grafting and microanastomosis procedures may result in inadvertent twisting of the grafts or native arteries. Twist alters both the wall stress distribution and blood flow, thus sustained twist affects reendothelialization and stimulates thrombosis and intimal hyperplasia.^{2, 9, 34, 41} Severe twist can cause buckling and kinking in these vessels^{8, 41} and can affect the patency of microvascular anastomoses.^{24, 39} In addition, twist in tissue engineered vascular grafts can alter their wall stress and thus the wall remodeling and mechanical properties. Alterations in mechanical properties could affect the patency of the vascular grafts.^{35, 40} Therefore, it is of clinical interest and need to determine how arteries remodel in response to axial twist.

There have been several reports of the mechanical behavior of arteries under torsion. In a pioneering work, Deng and colleagues designed a delicate torsion device and measured the shear modulus of rat arteries.⁷ Later, Lu and colleagues measured the shear modulus of porcine coronary arteries.³³ Based on further analysis of these data, Van Epps and Vorp determined the shear constant of the Fung strain energy function of porcine coronary arteries.⁴⁴ Recently, our lab determined the shear constant of the Fung strain energy function for porcine carotid arteries and demonstrated that severe twist can lead to artery buckling and kink formation that can potentially block blood flow.^{13, 21} However, it remains unclear how arteries remodel in response to sustained axial twist.

Like lumen pressure and blood flow, axial twist contributes to the mechanical stresses in arteries. Changes in mechanical stress are well known to stimulate arterial wall remodeling. Despite numerous studies that have documented the arterial adaptive response to changes in pressure, flow rate, and axial stretch ratio both *in vivo* and *ex vivo*,^{1, 4, 5, 19, 25, 32, 38} little is known about the arterial adaptive response to axial twist.

Accordingly, the objective of this study was to determine the early stage arterial wall remodeling in response to axial twist. We investigated the effect of axial twist on cell proliferation, endothelial morphology, and extracellular matrix (ECM) remodeling in porcine carotid arteries using an *ex vivo* organ culture model.

MATERIALS AND METHODS

Experimental Design

A porcine carotid artery organ culture model was used to examine wall remodeling in arteries under axial torsion. Arteries (about 40 mm in length) were subjected to a given twist angle of 180° while being cultured for 3 days under physiological pressure (100±20 mmHg), flow rate (160 ml/min) and axial stretch ratio (1.5), respectively.^{30, 31} A twist of 180° was chosen as it falls below the twist angle that may affect patency of the vessel reported in the literature^{24, 39} and it was easy to implement in organ culture. 180° is also the twist angle that occurs in vessels in the propeller flap skin grafting procedures used in skin grafting.⁴⁸ Due

to different protocol requirements, two sets of arteries were cultured under the same conditions, one set ($n=8$) for vascular cell proliferation, and protein measurements using immunohistochemistry and immunoblotting, the other ($n=6$) for endothelial cell (EC) morphology and orientation measurements using silver staining and *en face* microscopy for internal elastic lamina (IEL) fenestrae measurement. Each set had a twist group and a control group that were paired by using the collateral arteries from the same animals.

To distinguish the possible adaptation in EC morphology from deformation, another set ($n=4$) of arteries were cultured for 3 days under the same conditions, then used to examine the EC morphology after the twist was unloaded (untwist). A set of fresh arteries ($n=4$) were twisted and processed immediately for silver staining to capture the EC deformation due to the twist. Furthermore, a fifth set of arteries ($n=8$) were cultured under a reduced axial stretch ratio of 1.3 to detect the possible effects of twist on cell proliferation and matrix metalloproteinase under low axial stretch.

Artery Preparation and Ex Vivo Organ Culture

Bilateral porcine common carotid arteries were harvested from 6 to 7 month old farm pigs (100–150 kg) from a local slaughterhouse, rinsed with sterile PBS (Dulbecco's phosphate buffered saline, Sigma) and then transferred to our laboratory in ice cold PBS solution supplemented with 1% antibiotic-antimycotic (Gibco). Then, inside a Class II biosafety cabinet in our lab, arterial segments of 40–60 mm in length were prepared and checked for leaks with a brief inflation test.

All arteries were mounted into tissue chambers in *ex vivo* perfusion organ culture systems and maintained inside incubators (37°C, 5% CO₂) as described in detail previously.^{19, 22} The perfusion flow was pulsatile with a mean pressure of 100 mmHg (oscillating from 80 to 120 mmHg) and a mean flow rate of 160 ml/min. The distance between cannulae was adjusted to achieve the designated axial stretch ratios. The axial stretch ratio was calculated as the ratio of stretched vessel length between the two suture ties to its corresponding free length.

Axial Twist of Arteries

After incubating in the organ culture system overnight to recover from the effects of harvesting and initial operation, arteries of the twist group were twisted axially by rotating one end (with the cannula) 180° while the other end was fixed tightly. Suture ties were used as markers for measuring the twist angle. The pressure and flow rate in all the flow loops were fine tuned to the designated values and all arteries were then cultured for three days.

Cell Proliferation Labeling and Quantification

Bromodeoxyuridine (BrdU at 5 µg/ml, Sigma) was added to the perfusion medium 24 hours before the end of organ culture to label the nuclei of newly proliferated vascular cells. Anti-BrdU staining was carried out on 5 µm thickness frozen slides of the samples obtained following the protocol that has been used in our lab in previous studies.²² The cell nuclei were counterstained with 4', 6-Diamidino-2-Phenylindole, Dihydro-chloride (DAPI, Molecular Probes). The slides were examined via fluorescent microscopy and photographed.

The cells located in the intima and media were distinguished based on their location relative to IEL.⁴⁹ The number of BrdU-positive cells in the intima and media were counted respectively using Image-Pro Plus as previously described.¹⁷ BrdU index, the percentage of BrdU-positive cells, was calculated to quantify cell proliferation.

Immunoblotting

Arteries were harvested, washed three times with PBS, cut into segments of 4–6 mm in length, grinded on ice, and then stored at –70°C. Later, the samples were homogenized using the reagent 4X protein extraction buffer (Sigma). Protein extracts were separated by 10%–15% SDS-PAGE and transferred to PVDF membranes (Bio-Rad) and incubated with the one of the following primary antibodies: matrix metalloproteinase-2 (MMP-2) (1:500, Millipore), MMP-9 (1:300, Millipore), tissue inhibitor of metalloproteinase-2 (TIMP-2) (1:3000, sigma), glyceraldehydes 3-phosphate dehydrogenase (GAPDH) (1:3000, sigma). Protein bands were then visualized with the enhanced chemiluminescence (ECL, Amersham GE healthcare) detection system according to the manufacturer's directions. The relative photometric intensities of the protein bands were quantified using ImageJ.

Silver Staining and Characterization of EC Shape and Alignment

To visualize the contour of ECs in arteries under twist, arteries were kept in the tissue chamber under the same pressure and twist loads as during organ culture, and stained with silver nitrate following the protocol previously described.^{23, 32} Briefly, the lumen of the artery was washed with a perfusion of PBS and fixative 2% PFA (paraformaldehyde). Then, the arteries were perfused with PBS for 1 minute; 5% dextrose in DD water for 40 seconds; 0.25% AgNO₃ in DD water for 30 seconds; 5% dextrose in DD water for 40 seconds; and then fixative for 2 minutes. The perfusion flow rate and pressure were set to the same as that used during organ culture (160 mL/min, 100 mmHg) to avoid EC shape changes during silver staining.

To visualize the ECs after removal of torsion, arteries were cultured for 3 days under the same condition and removed from the organ culture system. The arteries were then stained with silver nitrate following the protocol previously described.³² Briefly, ring segments (~10 mm) were cut open longitudinally and pinned down with the endothelial surface upward, stretching to their axial length and perimeter in organ culture. Then *en face* staining was done with silver nitrate as described above.³² Afterwards the specimens were fixed for 24 hours under light.

For all specimens that underwent silver staining, EC shape and alignment were examined via *en face* light microscopy. ECs were photographed at several (4–6) fields for each specimen. The outlines of ECs were traced, and the morphological parameters such as area, major and minor axis lengths, perimeter length, and major axis direction were measured using ImagePro Plus 7.0. Cell morphology was quantified by shape index and aspect ratio:

$$\text{Shape Index} = (\text{Perimeter})^2 / 4\pi * (\text{Area}) \quad (1)$$

$$\text{Aspect Ratio} = (\text{Major Axis Length}) / (\text{Minor Axis Length}) \quad (2)$$

For each artery sample, about 300 ECs were measured and the mean value was calculated to represent the value for the specimen.

EC alignment was described by the angle between the major axis with respect to the flow direction. These angles were represented as a histogram with 36 bins and fitted to a Von Mises distribution wrapped between -90 and 90 degrees. The measure of central concentration k value for the Von Mises distribution, which is a reciprocal measure of dispersion ($1/k$ is analogous to σ^2 of the wrapped normal distribution), was determined using Matlab.⁴⁵ A k value of zero represents a uniform distribution, while a larger k value represents a distribution that is highly concentrated at the mean angle.

Laser Scanning Confocal Microscopy

The internal elastic lamina (IEL) was examined in a group of twisted arteries and controls following a protocol described in detail previously.⁵⁰ Briefly, artery segments (~ 10 mm length) were cut open longitudinally and stretched bi-axially to their dimensions under load (a stretch ratio of 1.5 longitudinally and 1.25 circumferentially, respectively) and then pinned down to a plate with the lumen side up. The specimens were then fixed in 4% formalin overnight and then treated with 0.5% triton X-100 for 15 minutes. Cell nuclei were stained with DAPI (1 $\mu\text{g/mL}$ Molecular Probes) for 30 minutes. The specimens were mounted under cover slips with Fluoromount-G (SouthernBiotech) for *en face* confocal microscopy (LSM 710, Zeiss, Germany). From each sample, five view fields were imaged under a 40x oil immersion objective. Later, the aspect ratio, size and area percentage of the fenestrae were quantified for these images using IMAGE PRO PLUS 7.0.

Determination of Shear Strain and Principal Strain Direction

The strains in the arteries were analyzed based on an axi-symmetric cylindrical vessel model to quantify the shear strain in the twisted arteries. We designate the initial inner radius, outer radius, and length of the artery at free condition to be R_i , R_e , and L , respectively. Similarly, the inner radius, outer radius, and stretched length of the artery at the pressure-loaded condition are designated as r_i , r_e and l , respectively. The artery is under the combined loads of axial force (N), lumen pressure (P_i), and torque (T) which twists the artery by a rotation angle (Φ). Accordingly, the average local shear strain (angle, β) in the lumen surface is¹³

$$\beta = \frac{\Phi r_i}{l} \quad (3)$$

Using cylindrical coordinates, a point with coordinates (R, Θ, ζ) at no load condition deforms to location (r, θ, z) in the loaded configuration. The deformation gradient matrix (F) for the artery under torsion is:

$$[F] = \begin{bmatrix} \frac{\partial r}{\partial R} & 0 & 0 \\ 0 & \frac{r}{R} & r\gamma \\ 0 & 0 & \lambda_0 \end{bmatrix} \quad (4)$$

Where, twist ratio $\gamma = \Phi/L$ and λ_0 is the axial stretch ratio. Accordingly, the Green's strain tensor (E) can be expressed as

$$E = \frac{1}{2} \begin{bmatrix} \left(\frac{\partial r}{\partial R}\right)^2 - 1 & 0 & 0 \\ 0 & \left(\frac{r}{R}\right)^2 - 1 & \frac{r^2\gamma}{R} \\ 0 & \frac{r^2\gamma}{R} & (r\gamma)^2 + (\lambda_0)^2 - 1 \end{bmatrix} \quad (5)$$

To determine the principal direction (angle α) in the vessel wall surface plane (θ - z), the eigenvector of the sub-matrix below was determined.

$$E_{2D} = \frac{1}{2} \begin{bmatrix} \left(\frac{r}{R}\right)^2 - 1 & \frac{r^2\gamma}{R} \\ \frac{r^2\gamma}{R} & (r\gamma)^2 + (\lambda_0)^2 - 1 \end{bmatrix} \quad (6)$$

Statistical Analysis

Data are presented as mean $\pm$ SEM (standard error of the mean) unless otherwise noted. Statistical significance was determined using a 1-way ANOVA test followed by a Student-Newman-Keuls post hoc test for the comparisons of means, or a paired t -test when only two means were compared. A value of $p < 0.05$ was set as statistically significant.

RESULTS

Cell Proliferation

New proliferating vascular cells were observed in all arteries after 3 days in organ culture (Fig. 1). The cell proliferation indexes in both intima and media layers were significantly higher ($p < 0.05$) in the twist groups than in the control groups, under both the low and normal axial stretch ratios. Meanwhile, there was no statistically significant difference between the two control groups under the two stretch ratios. These results suggested that the difference in cell proliferation was not due to axial stretch ratio but the twist.

Internal Elastic Lamina (IEL) Remodeling

Reduced IEL fenestrae total area, reduced mean fenestrae size, and elongated fenestrae shape were observed in the twist arteries under the normal axial stretch ratio of 1.5 (Fig. 2). The percentage of fenestrae to total IEL area ratio was significantly decreased by nearly half in the twist group compared to the control group ($p < 0.01$). The average size of individual fenestrae was significantly decreased in the twist group by nearly half as well ($p < 0.01$). Meanwhile, the average aspect ratio of the fenestrae was significantly increased from 2.39 ± 0.15 in control groups to 2.83 ± 0.29 in twist groups ($p < 0.05$), indicating significantly elongated shape after 3 days under twist.

Adaptation of EC Shape and Alignment

ECs in the twisted arteries adapted their shape and alignment during organ culture. After 3 days organ culture, the twisted arteries showed significantly elongated shape compared with the controls. Both aspect ratio and shape index of the twisted arteries cultured for 3 days were significantly increased compared with the controls (Fig. 3).

The alignment of ECs in the twisted arteries also adapted to be closer towards the flow direction. Initially, when the arteries were twisted at the beginning of organ culture, the ECs aligned towards an angle of $15.6 \pm 1.3^\circ$ with the twist deformation. After 3 days in organ culture the alignment angle of the twist arteries reduced to $7.5 \pm 2.4^\circ$ ($p < 0.05$, Fig. 4). Furthermore, the alignment angle in the additional set of twisted arteries measured after removal of the twist was $-8.2 \pm 3.7^\circ$ ($p < 0.05$, Fig. 4). Since the arteries recovered to their original configuration after the twist load was removed, this negative angle further demonstrated that the EC alignment angle was reduced (from the initial value) in these twisted arteries before unloading. In contrast, the alignment angles of all the control groups showed no significant difference after organ culture. In addition, the k value showed that the ECs alignment was significant in both control and twist arteries ($p < 0.05$), although the angle was more concentrated after 3 days organ culture in the twist group.

Effect of Axial Twist on MMP-2, MMP-9, TIMP-2

MMP-2 expression was significantly increased in the twist group compared to the control group for arteries cultured for 3 days under both axial stretch ratios ($p < 0.05$, Fig. 5 and Fig. 6). However, TIMP-2 and MMP-9 showed no statistical differences in the control and twist groups, respectively.

Principal Strain Direction in the Twisted Arteries

Using the dimensions of the fresh arteries used in silver staining, the shear strains in the twisted arteries were estimated to be $\beta = 9.4 \pm 0.8^\circ$ and the principal strain directions were determined to be $\alpha = 4.6 \pm 0.5^\circ$ from the axial (flow) direction.

DISCUSSION

In this study, we investigated the artery remodeling resulting from axial twist using an *ex vivo* organ culture model. Our results demonstrated that subjecting arteries to axial twist for 3 days increased cell proliferation in both the intima and media and increased MMP-2 expression in the arterial wall. Axial twist also significantly reduced the fenestrae size and made them more elongated. In addition, axial twist significantly elongated the ECs as measured by the shape index and aspect ratio, and aligned the ECs toward the direction of the principal strain.

Increased cell proliferation plays an important role in the pathogenesis of neo-intimal hyperplasia and atherosclerosis.¹¹ The decrease in fenestrae size and total area ratio, which are believed to relate to the wall permeability, could increase the resistance to macromolecular transportation across the IEL and could potentially increase macromolecular accumulation in the intima potentially triggering atherosclerosis.^{16, 43}

It is well accepted that the ECM of the arterial wall remodels in response to mechanical stress and that the process is modulated by matrix metalloproteinases MMP-2 and MMP-9. On the other hand, TIMP-2 inhibits MMP-2 activity.^{26, 27, 42} Although we did not examine MMP activity using zymography, our results showed that the expression of MMP-2 significantly increased in the twist group while there was no significant change of TIMP-2. The increase in the MMP2 to TIMP-2 ratio indicates increased ECM remodeling in the twisted arteries, which is often associated with pathological changes in the development of cardiovascular diseases such as atherosclerosis, plaque instability.¹² We did not measure the MMP expression in fresh groups because we assumed that changes in MMPs did not occur right after wall stress is increased.¹⁵ Compared to deformation, changes in MMP expression and structural remodeling occur over time and our previous studies demonstrated no significant change in MMPs in the 3-day cultured control group compared to the fresh group.²²

ECs in arteries are exposed to both fluid shear stress produced by blood flow and periodic stretching generated by pressure and axial tension. The morphology and cytoskeleton microstructure of ECs directly affects their function.²⁹ It is well known that EC orientation and shape are influenced by shear flow, cyclic circumferential stretch, and axial stretch.^{6, 29, 32} EC morphology has been related to local shear stress patterns in the vasculature.¹⁰ ECs tend to elongate and align in the direction of flow but perpendicular to cyclic stretch.^{47, 51} Here, we showed that ECs significantly elongate in response to twist deformation. Since the vessel deformation can result in cell deformation, we compared EC morphology between the twist and control for fresh arteries. ECs align initially in a diagonal direction of $\sim 15^\circ$ under axial twist. After 3 days in organ culture, the EC realign partially back towards the (axial) flow direction ($\sim 7^\circ$), indicating EC adaptation. This adaptation over time is most likely due to the fluid flow in the lumen. However, it is not clear whether the remaining angle, the difference in alignment angles between the twist and control arteries after 3-day organ culture was due to the limited time duration or due to the change in the principal strain direction. While future work is needed to better quantify the local strains and to determine the adaptation of ECs under long-term twist, our results clearly demonstrate that EC shape and alignment adapted to the axial twist of arteries.

The increases in cell proliferation, and MMP-2 expression as well as the changes in EC and IEL fenestrae morphology due to axial twist are similar to the features of the remodeling process that occurs in response to elevated axial stretch and pulse pressure.^{14, 18, 19, 22, 25} These results suggest that the shear stress generated by axial twist has similar biological effects compared to increased normal stress generated by elevated pressure or axial stretch. Thus, these results broaden our understanding of stress-induced wall remodeling by including torsional wall shear stress.

While the local twist in the carotid arteries is normally small during head rotation, implantation of stents could lead to increased local twisting and bending at the ends of the stent that could be aggravated by head movement, potentially leading to long-term stent failure.^{37, 46} However, very little attention has been focused on the biological adaptive response of arteries to twist. Our results identify the features of early stage arterial

remodeling under twist, which could help understand the adaptation of stented carotid arteries.

One advantage of the use of the *ex vivo* organ culture model was that it allowed us to apply an axial twist to the arteries without altering the mean pressure, flow rate, or axial stretch ratio in a well-controlled environment. One limitation of the organ culture model is the limited duration of observation. Previous reports from multiple groups including ours have demonstrated that organ culture maintains the structure and function of arteries for 3, 7, or 9 days.^{18, 22, 36} The fact that the EC morphology showed no difference between fresh and 3 days controls further validated that organ culture itself did not change EC morphology. The long-term adaptation of the arteries under twist needs to be investigated in the future using animal models.

Arteries are often subject to axial twist due to body movement or surgical interventions. Excessive twisting can disrupt blood flow and trigger thrombosis and atherosclerosis.^{24, 39} Our results demonstrate that arterial wall remodeling does occur in response to axial twist. These results shed light on the mechanisms of arterial wall remodeling in twisted vessels and are complimentary to knowledge of arterial wall remodeling due to changes in lumen pressure, flow, and axial stretch.

CONCLUSIONS

Axial twist of arteries affects EC morphology and stimulates cell proliferation and ECM remodeling in the arterial wall. Our results suggest that both normal stress and shear stress similarly affect arterial wall remodeling. These results enrich our understanding of the early stage artery remodeling in response to alterations in the mechanical environment.

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FIGURE 1.

Photographs: Representative images of BrdU stained regions of artery samples for control and twist groups under low and normal stretch ratios (1.3 and 1.5, respectively). The lumen side of the vessel is upward in these images. Scale bar = 100 μ m. Bargraphs: Comparison of BrdU indices for the cells in intima and media of arteries from control and twist groups under low and normal stretch ratio. For the low stretch ratio groups, $n=5$. For the normal stretch ratio groups, $n=6$. * $p < 0.05$ versus the controls.

FIGURE 2.

Photographs: Representative confocal images of fenestrae (marked by arrows) in the IEL of cultured arteries from the control and twist groups under normal stretch ratio (stretch ratio = 1.5). Scale bars represent 15 μm . Bargraphs: Comparison of total fenestrae area ratio, individual fenestrae size, and fenestrae aspect ratio of arteries in the control and twist groups. For all groups, $n = 4$. ** $p < 0.01$, * $p < 0.05$.

FIGURE 3.

Top: Micrographs from *en face* light microscopy illustrating the outlines of ECs visualized by silver staining under normal stretch ratio (stretch ratio =1.5). Scale bar = 15 μ m. The arrows show the direction of flow. Bottom: Comparison of the EC aspect ratio and shape index in the control and twist groups cultured for 3 days. For all groups, n=4. * $p < 0.05$ vs controls; # $p < 0.05$ versus twist group of “3 days” (measured under twist).

FIGURE 4.

Top: Comparison of the EC alignment angles shows the realignments of EC orientation after 3 days organ culture. For all groups $n=4$. * $p < 0.05$ versus corresponding control; # $p < 0.05$ versus twist group of “3 days”(measured under twist). Bottom: EC alignment angle from the arteries which were stained under twist with the fitted von Mises distribution. The k value is the measure of central concentration.

FIGURE 5.

Photograph: Representative western blotting results show the levels of MMP-2, Timp-2, MMP-9 and GAPDH in the control and twist artery groups under low stretch ratio (stretch ratio =1.3). Bargraphs: Comparison of the relative intensities of MMP-2, TIMP-2, MMP-9 bands of the control and twist group. MMP-2 was significantly increased due to twist, while MMP-9 and TIMP-2 showed no measurable significant difference. For all groups n=7. * $p < 0.05$.

FIGURE 6.

Photograph: Representative western blotting results show the levels of MMP-2, Timp-2, MMP-9 and GAPDH in the control and twist artery groups under normal stretch ratio (stretch ratio =1.5). Bargraphs: Comparison of the relative intensities of MMP-2, TIMP-2, MMP-9 bands of the control and twist group. MMP-2 was significantly increased due to twist, while MMP-9 and TIMP-2 showed no measurable significant difference. For all groups n=6. * $p < 0.05$.