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# Uniaxial stretch-induced regulation of mitogen-activated protein kinase, Akt and p70S6 kinase in the ageing Fischer 344 × Brown Norway rat aorta

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**Experimental Physiology**

# Uniaxial stretch-induced regulation of mitogen-activated protein kinase, Akt and p70 S6 kinase in the ageing Fischer 344 × Brown Norway rat aorta

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**The effects of ageing on the cardiovascular system contribute to substantial alterations in cellular morphology and function. The variables regulating these changes are unknown; however, one set of signalling molecules that may be of particular importance in mediating numerous cellular responses, including control of cell growth, differentiation and adaptation, are the proteins associated with the mitogen-activated protein kinase (MAPK) signalling systems. The MAPKs, in conjunction with the p70 S6k signalling cascade, have emerged as critical components for regulating numerous mechanotransduction-related cellular responses. Here we investigate the ability of uniaxial stretch to activate the MAPK and p70 S6k pathways in adult (6-month-old), aged (30-month-old) and very aged (36-month-old) Fischer 344/NNiaHSd × Brown Norway/BiNia (FBN) rats. Western blotting of the MAPK family proteins extracellular signal-regulated kinase (Erk) 1/2, p38- and c-Jun $NH_2$-terminal kinase (Jnk)-MAPKs showed differential expression and activation between these proteins with age. An acute 15 min interval of 20% uniaxial stretch using an *ex vivo* aortic preparation demonstrated similar regulation of Erk1/2, p38- and Jnk-MAPK. However, ageing altered uniaxial induced p70 S6k pathway signalling. These observations confirm previous data demonstrating that MAPK proteins are mechanically regulated and also suggest that p70 S6k signalling expression and activation are controlled differently with ageing. Taken together, these data may help to explain, in part, the age-related changes in vascular morphology, function and response to injury.**

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Recent studies have suggested that abnormal cellular responses to mechanical stress may play a role in the development of many diseases, including asthma, atherosclerosis, diabetes, stroke and heart failure (Yu *et al.* 1999; Abedin *et al.* 2004; Libby & Theroux, 2005). Ageing is a major risk factor for the development of cardiovascular disease (CVD), which is the leading cause of death in individuals over the age of 65 years (Lakatta, 2002). It is thought that the impact of age on the occurrence, severity and prognosis of CVD is due, in part, to age-associated changes in cardiovascular structure and/or function. However, this hypothesis has not been well studied (Sumitani *et al.* 1997). In the cardiovascular system, chronic stretch of vascular smooth muscle (VSMC) cells is directly associated with intimal–medial thickening *in vivo* (Jiang *et al.* 2000), the latter being the primary risk factor associated with cardiovascular disease. It has been hypothesized that this adaptive response is mediated by mechanosensing mechanism(s). However, neither the biochemical signalling involved in the adaptive response nor the effects of ageing on this process are well understood.

The effect of static and cyclic multiaxial strain on smooth muscle cells has been examined in several studies and pathways including G-protein-coupled receptors, ion channels, mitogen-activated protein kinases (MAPK), protein kinase B (Akt/PKB), protein kinase C (PKC), nuclear factor $\kappa$B, RhoA/Rho-kinase and several others

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have been implicated in transduction of the mechanical stimulus and the subsequent cellular response (Nakayama *et al.* 2003; Lee *et al.* 2004; Lehoux *et al.* 2006). The MAPK signalling in response to uniaxial strain has been shown to be altered depending upon direction of stretch in a smooth muscle cell (SMC) culture model (McKnight & Frangos, 2003). One study has reported a higher matrix metalloprotease (MMP) activation in cultured SMC subjected to static uniaxial compared with cyclic stretch (Asanuma *et al.* 2003). However, the distinction between the smooth muscle signalling in response to uniaxial stretch and multiaxial stretch is not well understood. Wang and Thempatty in their recent review of cell mechanobiology have expressed a need for more studies delineating cellular mechanical sensors and response mechanisms in different types of stretch (Wang & Thampatty, 2006). In endothelial cells, directed mechanical forces such as sustained uniaxial stretch have been shown to cause a transient upregulation of pro-inflammatory and proliferative cytokines with subsequent downregulation even if the forces are sustained. In contrast, sustained multiaxial stretch leads to sustained upregulation of these pathways (Chien, 2007). Striated muscle cells demonstrate the capacity to distinguish between different types of mechanical signals (Kumar *et al.* 2004). Taken together, these studies suggest that the activation of intracellular signalling pathways in skeletal, endothelial and even cultured smooth muscle cells may be specific to the type (uniaxial *versus* multiaxial) of mechanical force applied. However, the effect of directional loading on differentiated smooth muscle has not been examined in depth. Also, the impact of ageing, a major risk factor for cardiovascular disease, on the capacity of blood vessels to differentiate between various mechanical stimuli and to initiate appropriate signalling cascades is not well understood.

We have previously demonstrated an effect of ageing on the contractile and mechanical properties of the Fischer 344/NNia × Brown Norway/BiNia (FBN) rat aorta (Blough *et al.* 2006). Using segments of the rat aorta that had been subjected to a static multiaxial stretch, we have reported that this type of loading is capable of activating the MAPK and p70 S6k pathways and that the activation of these pathways is altered with ageing (Rice *et al.* 2005*a*,*b*). Here we examine the intracellular signalling pathways following a uniaxial stretch stimulus in segments obtained from the same aortae used in the multiaxial stretch experiments. We hypothesized that the phosphorylation of Erk1/2 and Akt, but not p70 S6k, would be increased in smooth muscle by uniaxial stretch. Using our previous studies as a guide, we also hypothesized that the magnitude of stretch-induced Erk1/2, Akt and p70 S6k would be altered with ageing. Considered in combination with our previous data (Rice *et al.* 2005*a*,*b*), the results of the present investigation suggest for the first time that differentiated smooth muscle, similar to striated muscle as well as to cultured SMC and endothelial cells, exhibits the ability to differentiate between different types of stretch stimuli. Furthermore, we show that ageing differentially affects the ability of the aorta to activate the MAPK and p70 S6k pathways in response to a uniaxial stretch stimulus.

## Methods

### Animals

All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals as approved by the Council of the American Physiological Society and the Animal Use Review Board of Marshall University. All procedures were conducted in strict accordance with Public Health Service animal welfare policy. Adult (6 month), aged (30 month) and very aged (36 month) male Fischer 344/NNiaHSd × Brown Norway/BiNia F1 hybrid rats, obtained from the National Institute for Ageing, were housed two per cage in an AAALAC approved vivarium. Housing conditions consisted of a 12 h–12 h light–dark cycle and temperature was maintained at $22 \pm 2^{\circ}$C. Animals were provided with food and water *ad libitum*. Rats were allowed to recover from shipment for at least 2 weeks before experimentation, during which time the animals were carefully observed and weighed weekly. Any of the rats showing signs of failure to thrive, such as precipitous weight loss, disinterest in the environment or unexpected gait alterations, were excluded from the study.

### Materials

Antibodies against p-p44/p42 MAPK (Thr 202/Tyr 204; mitogen-activated protein kinase ERK1/2; no. 9106), p-p38 MAPK (Thr 180/Tyr 182; no. 9216), p-SAPK/Jnk (Thr 183/Tyr 185; no. 9251), p-p70 S6k (Thr 389; no. 9205), p-p70 S6k (Thr 421/Ser 424; no. 9204), p-GSK-3$\beta$ (Ser 9; (glycogen kinase-3$\beta$; no. 9336), p-Akt (Thr 308; no. 9275), p-Akt (Ser 473; no. 9271), p-SHP-2 (Tyr 452; Src-homology domain 2-containing tyrosine phosphatase; no. 3751), and p-PTEN (Ser 380/Thr 382/383; (phosphatase and tensin homologue; no. 9554) mouse IgG and rabbit IgG antibodies were purchased from Cell Signalling Technology (Beverly, MA, USA). Precast 10% SDS-PAGE gels were procured from Cambrex Biosciences (Baltimore, MD, USA). Enhanced chemiluminescence (ECL) Western blotting detection reagent was from Amersham Biosciences (Piscataway, NJ, USA). Restore Western blot stripping buffer was obtained from Pierce (Rockford, IL, USA) and 3T3 cell lysates were from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

All other chemicals were purchased from Sigma (St Louis, MO, USA).

### Blood vessel preparation

Rats were anaesthetized with a ketamine (40 mg kg$^{-1}$) xylazine (10 mg kg$^{-1}$) cocktail I.P. and supplemented as necessary for reflexive response. Aortae were isolated and removed as outlined elsewhere (Rice *et al.* 2005*b*). Once excised, a portion of the vessel was cut longitudinally with a sharp razor and the vessel segment attached to a micro-calliper with ethyl cyanoacrylate along the longitudinal cut. The micro-calliper with the mounted vessel was attached to a force transducer connected to an adjustable stage. The stage was adjusted to provide a force reading of 0 g. The *in situ* resting length was taken as the length of 0 g of force. After mounting, aortae were allowed to equilibrate in the vessel chamber for at least 1 h before stretch loading. The vessels were then stretch to 20% of their *in situ* resting length for a period of 15 min. Vessel manipulation was done in oxygenated Krebs–Ringer bicarbonate (KRB) buffer maintained at 37°C.

### Immunoblotting

Sample preparation and immunoblotting were performed as detailed previously (Rice *et al.* 2005*a*,*b*). Briefly, aortae were lysed on ice for 30 min in tissue protein extraction reagent (T-PER; Pierce, Rockford, IL, USA) supplemented with 100 mM NaF, 1 mM $Na_3VO_4$, 2 mM phenylmethylsulfonyl fluoride (PMSF), 1 $\mu$g ml$^{-1}$ aprotinin, 1 $\mu$g ml$^{-1}$ leupeptin and 1 $\mu$g ml$^{-1}$ pepsatin and the supernatant collected by centrifugation (10 min at 800*g*). Protein concentration was determined in triplicate using the Bradford method (Pierce) and 30 $\mu$g of protein were separated using 10% SDS-PAGE gels. Transfer of protein onto nitrocellulose membranes was performed using standard conditions and verified by staining with Ponceau S. Immunodetection of antigens was performed as previously described (Rice *et al.* 2005*a*,*b*). To allow direct comparisons to be made between the concentration levels of different signalling molecules, immunoblots were stripped and reprobed with Restore Western blot stripping buffer as detailed by the manufacturer. After verifying the absence of residual HRP activity on the membrane by treating the membrane with the ECL reagent, membranes were washed and reprobed.

### Data analysis

Results are presented as means ± S.E.M. Data were analysed by using SigmaStat 3.0. Multiple group comparisons were performed by one-way ANOVA followed by Student–Newman–Keuls *post hoc* test used to determine differences between groups. The level of significance accepted *a priori* was $P = 0.05$.

## Results

### Verification of loading stimulus

Isolated tissue preparations responded to stretch in a passive manner. To examine whether the loading stimulus was constant throughout the loading procedure, we constantly recorded system tension of the mounted aorta. If fluctuations in stretch-induced loading occurred, the vessel was immediately discarded.

### Stretch-induced MAPK phosphorylation is altered in the ageing rat aorta

The effect of ageing on the amounts and phosphorylation of Erk1/2, p38-MAPK, and Jnk has been previously reported (Rice *et al.* 2005*a*). In aortae subjected to 20% uniaxial stretch, the phosphorylation of Erk1 (p44 MAPK; Thr 202/Tyr 204) was increased by 77.2 ± 6.8, 67.1 ± 14.0 and 25.0 ± 13.6% in the 6-, 30-, and 36-month-old aortae, respectively ($P < 0.05$; Fig. 1). Stretch-induced phosphorylation of Erk2 (p42 MAPK; Thr 202/Tyr 204) responded similarly to Erk1, demonstrating increases of 132.1 ± 5.1, 78.8 ± 17.7 and 78.2 ± 15.3% in the

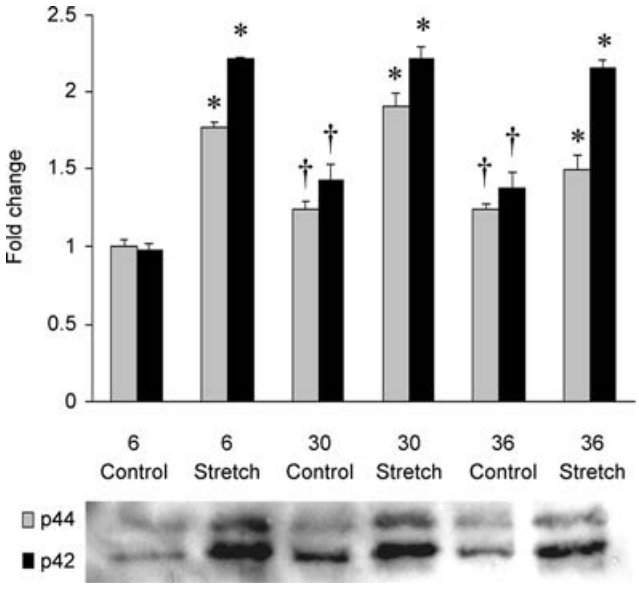


**Figure 1. The basal (control) and stretch-induced phosphorylation of the Erk1/2 (p44/p42; Thr 202/Try 204) in aortae from 6-, 30- and 36-month-old rats**
Immunoblot analysis for p44 and p42 phosphorylated at Thr 202/Try 204. Values are expressed relative to 6-month-old control values. * Significant difference from the corresponding control value; † significant difference from the corresponding 6 month value ($P < 0.05$); $n = 4$ per group.

6-, 30-, and 36-month- old aortae, respectively ($P < 0.05$; Fig. 1). In a similar fashion, 20% uniaxial stretch increased the phosphorylation of p38$\alpha$ (Thr 180/Tyr 182) by $251.4 \pm 51.8$, $409.6 \pm 30.3$ and $273.7 \pm 52.8$% in the 6-, 30-, and 36-month old aortae, respectively ($P < 0.05$; Fig. 2). The p38$\gamma$ (Thr 180/Tyr 182) phosphorylation increased in response to a 20% uniaxial stretch by a magnitude of $312.6 \pm 59.1$, $492.2 \pm 29.1$ and $353.9 \pm 75.3$% in the 6-, 30-, and 36-month-old aortae, respectively ($P < 0.05$; Fig. 2). The Jnk1 (Thr 183/Tyr 185) phosphorylation was also elevated in response to a 20% uniaxial stretch, demonstrating increases of $106.8 \pm 8.5$, $91.2 \pm 7.7$ and $95.2 \pm 11.4$% in the 6-, 30-, and 36-month-old aortae, respectively ($P < 0.05$; Fig. 3). Similarly, Jnk3 (Thr 183/Tyr 185) phosphorylation increased by $87.6 \pm 14.5$, $113.25 \pm 8.8$ and $95.2 \pm 12.9$% in the 6-, 30-, and 36-month-old aortae, respectively ($P < 0.05$; Fig. 3). Phosphorylation of Jnk2 (Thr 183/Tyr 185) was unchanged with stretch (Fig. 3).

### Uniaxial stretch-induced p70 S6k pathway phosphorylation is altered in the ageing rat aorta

The effect of ageing on the amount and phosphorylation of p70 S6k, GSK-3$\beta$, Akt, SHP-2 and PTEN expression in the ageing FBN aorta has been previously reported (Rice *et al.* 2005*b*). In aortae subjected to an acute 20% uniaxial stretch, the mTOR-dependent phosphorylated form of p70 S6k (Thr 389) was decreased by $58.4 \pm 6.9$, $24.8 \pm 7.8$ and $84.7 \pm 22.7$% in the 6-, 30- and 36-month-old aortae, respectively ($P < 0.05$; Fig. 4). Similarly, the Erk1/2-dependent form of phosphorylated p70 S6k (Thr 421/Ser 424) decreased by $51.3 \pm 15.1$, $48.7 \pm 6.0$ and $32.6 \pm 12.0$% in the 6-, 30- and 36-month-old aortae, respectively ($P < 0.05$; Fig. 4). Stretch appeared to affect the phosphorylation status of the upstream p70 S6k regulator Akt differently depending upon the residue examined. The Akt (Ser 308) was dephosphorylated by $24.7 \pm 10.7$, $30.9 \pm 9.1$ and $36.3 \pm 5.5$% in the 6-, 30- and 36-monthold aortae, respectively, with stretch ($P < 0.05$; Fig. 4) while, in contrast, stretch increased the phosphorylation of Akt (Ser 473) by $48.7 \pm 6.0$ and $32.6 \pm 12.0$% in 6- and 30-month old aortae, respectively ($P < 0.05$; Fig. 4). No significant response to the 20% uniaxial stretch was detected in the 36-month-old age group (Fig. 4). Uniaxial stretch increased the Akt-dependent form of phosphorylated GSK-3$\beta$ (Ser 9) in the 6-, 30-, and 36-month-old aortae by $83.1 \pm 13.0$, $21.9 \pm 10.8$ and $110.7 \pm 14.0$%, respectively ($P < 0.05$; Fig. 4) while SHP-2 phosphorylation was unaltered (Fig. 4). In contrast, phosphorylation of PTEN was decreased in response to uniaxial stretch loading by $60.1 \pm 3.9$, $78.9 \pm 3.3$ and $25.6 \pm 3.1$% in the 6-, 30- and 36-month-old aortae, respectively ($P < 0.05$; Fig. 4).

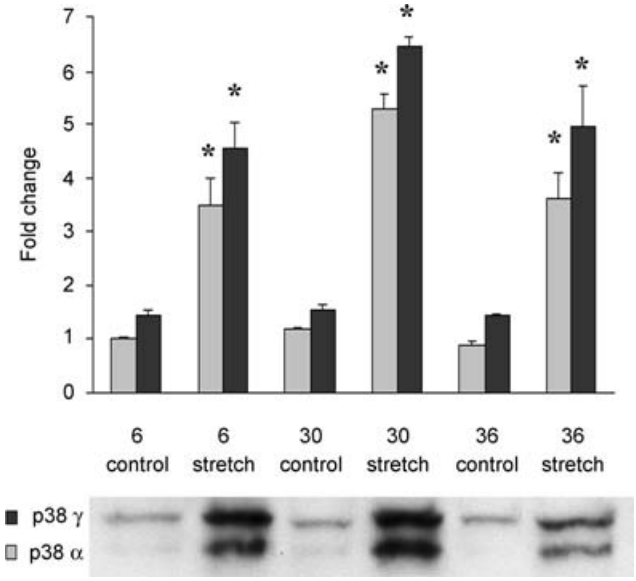


**Figure 2. The basal (control) and stretch-induced phosphorylation of the p38$\alpha/\gamma$ (Thr 180/Try 182) in aortae from 6-, 30- and 36-month-old rats**
Results were obtained by immunoblot analysis for p38$\alpha/\gamma$ phosphorylated at Thr 180/Try 182. Values are expressed relative to 6-month-old control values. * Significant difference from the corresponding control value; † significant difference from the corresponding 6 month value ($P < 0.05$); $n = 4$ per group.

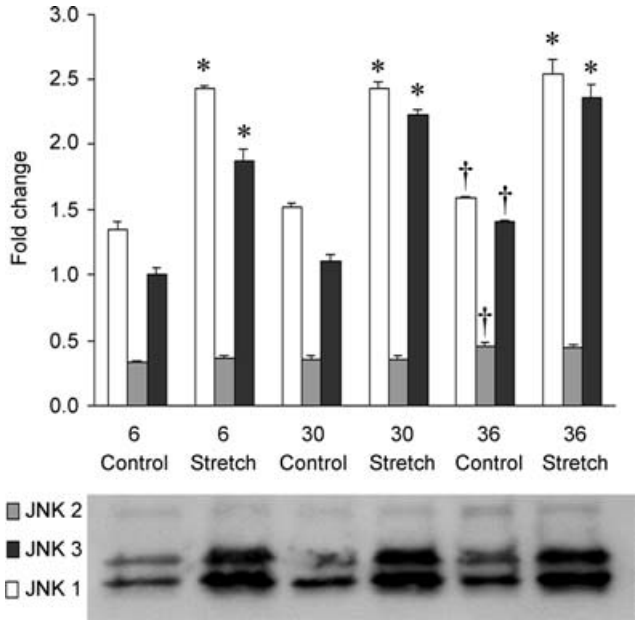


**Figure 3. The basal (control) and stretch-induced phosphorylation of the Jnk1/2/3 (Thr 183/Try 185) in aortae from 6-, 30- and 36-month-old rats**
Results were obtained by immunoblot analysis for Jnk1, Jnk2 and Jnk3 phosphorylated at Thr 183/Try 185. Values are expressed relative to 6-month-old control values. * Significant difference from the corresponding control value; † significant difference from the corresponding 6 month value ($P < 0.05$); $n = 4$ per group.

## Discussion

The MAPK pathways have been identified as important signalling proteins involved in the control of cell growth, differentiation and adaptation (Tibbles & Woodgett, 1999; Heo & Han, 2006). It has previously been demonstrated that multiaxial stretch is a potent activator of the MAPK pathway in a variety of cell types (Hellstrand & Albinsson, 2005; Nguyen *et al.* 2006). The effect of various stretch types is shown to have a varying effect on mesenchymal stem cell differentiation to smooth muscle cells (Park *et al.* 2004). However, the role of MAPKs in smooth muscle cell adaptive response to uniaxial stretch remains unknown. In the present study, we show that uniaxial stretch increases the phosphorylation of Erk1/2, p38-MAPK and Jnk in the aorta (Figs 1, 2 and 3). These findings are in agreement with others reporting the mechanical regulation of these proteins in striated muscle and other cell types (Tibbles & Woodgett, 1999; Li & Xu, 2000; Hellstrand & Albinsson, 2005; Abdulnour *et al.* 2006; Nguyen *et al.* 2006). Similarly, when considered in conjunction with previous studies in striated muscle (Kumar *et al.* 2002) and vascular smooth muscle cell culture (McKnight & Frangos, 2003), as well as our own findings in differentiated smooth muscle (Rice *et al.* 2005*a*), these data suggest that stretch type (uniaxial *versus* multiaxial) may not be an important determinant of load-induced MAPK activation. Furthermore, consistent with what has been recently shown in striated muscle and multiaxial aortic loading (Rice *et al.* 2005*a*), the magnitude of the stretch-induced p38-MAPK phosphorylation appears to be greater than that seen for either the Erk1/2- or Jnk (Figs 1, 2 and 3). This suggests that p38-MAPK phosphorylation in muscle may be more 'mechanically sensitive' than the Erk1/2- or Jnk proteins. Whether p38-MAPK exhibits a similar heightened response to loading in other tissue or cell types is not well characterized.

The p70 S6k, a serine/threonine protein kinase, plays a central role in cell growth and proliferation by mediating the phosphorylation of the 40S ribosomal protein, S6, thereby enabling efficient translation of 5′-terminal oligopyrimidine tract mRNA species. There is a limited body of literature on the effect of various types of stretch on the activation of the p70 S6k pathway. Uniaxial stretch has been shown to upregulate matrix degradation by VSMC (Asanuma *et al.* 2003), and the p70 S6k pathway has been implicated in modulation of the extracellular matrix profile via MMP activation (Bradley *et al.* 2003) as well as SMC differentiation (Martin *et al.* 2004). However, the effect of such stretch on differentiated smooth muscle has not been examined. Recent *in vitro* and *in vivo* studies have suggested that the p70 S6k pathway plays an important role in the phenotypic adaptations of striated muscle to hypertrophic stimuli (Baar & Esser, 1999). This is important because members of this class of transcripts are involved in cell cycle progression and the translational machinery (e.g. elongation factors, ribosomal proteins; Jefferies *et al.* 1997; Dufner & Thomas, 1999). Illustrating this fact is the finding that blockade of p70 S6k activity results in a significant inhibition of protein synthesis in multiple cell systems (Jefferies *et al.* 1997; Dufner & Thomas, 1999; Mourani *et al.* 2004). Investigating the effects of stretch type (uniaxial *versus* multiaxial) on p70 S6k phosphorylation in cultured skeletal muscle myotubes, Hornberger *et al.* (2005) demonstrated that multiaxial, but not uniaxial stretch, was capable of inducing the phosphorylation of p70 S6k in an Erk1/2-dependent manner. We have previously shown that multiaxial stretch is a potent activator of p70 S6k in the rat aorta (Rice *et al.* 2005*b*). Conversely, in the present report we show that a uniaxial stretch diminishes the extent of Erk1/2 (Thr 421/Ser 424)-dependent p70 S6k phosphorylation (Fig. 4). Taken together, these data suggest that, similar to striated muscle, p70 S6k activation in differentiated smooth muscle is sensitive to the type of stretch stimulus.

Protein kinase B (PKB/Akt) phosphorylation, an upstream activator of p70 S6k, is known to function in multiple cellular signalling pathways, has been demonstrated to be particularly important in opposing apoptotic cell death in a variety of cell types (Vivanco & Sawyers, 2002) and is activated in response to mechanical stimuli in smooth muscle (Rice *et al.* 2005*b*). The present data show that uniaxial stretch increased Akt (Thr 473)

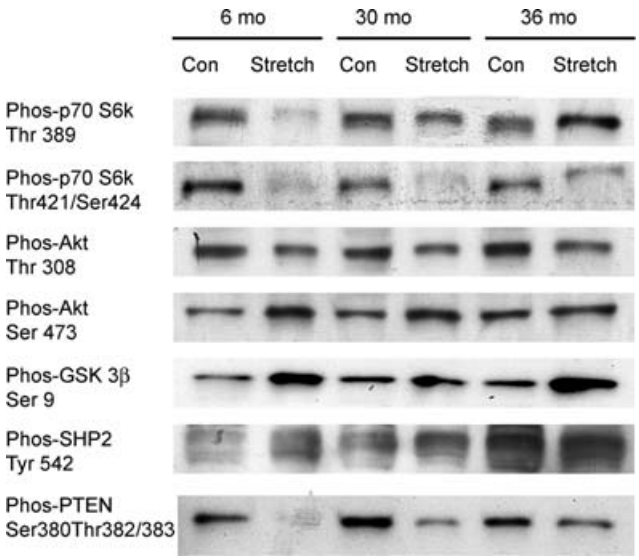


**Figure 4. Immunoblot analyses of basal (control) and stretch-induced phosphorylation of the mTOR-dependent form of p70 S6k (Thr 389), Erk1/2-dependent form of p70 S6k (Thr 421/Ser 424), Akt (Thr 308), Akt (Ser 473), GSK-3$\beta$ (Ser 9), SHP-2 (Tyr 542) and PTEN (Ser 380/Thr 382,383) in aortae from 6-, 30- and 36-month-old rats**

Please refer to values in Results ($n = 4$ per group; $P < 0.05$).

phosphorylation while, conversely, this stimulus resulted in a decrease in Akt (Thr 308) phosphorylation. These findings suggest that the stretch-induced activation of Akt in smooth muscle is dependent upon stretch type. The phosphatase activity of PTEN to inhibit the PI3K/Akt and Erk1/2-MAPK signalling cascades is thought to be negatively regulated by phosphorylation (Hlobilkova *et al.* 2003), and the inhibitory effect of uniaxial stretch on PTEN phosphorylation appears to antagonize Akt activation through the phosphoinositide kinase 3(PI3K) pathway. However, there may be a balance between SHP-2-mediated Akt activation and PTEN-mediated inactivation in response to uniaxial stretch, with a net effect of Akt activation. Also, feedback mechanisms leading to PTEN inactivation may already be activated at the time point examined in this study. The Ser 9 phosphorylation of GSK-3$\beta$ upon application of uniaxial stretch was found to be increased in all three age groups, even in the absence of Akt (Ser 473) phosphorylation in the 36-month-old rat aortas. It has been suggested recently that Akt may not be the exclusive regulator of GSK-3$\beta$ phosphorylation. For example, under certain conditions of growth factor removal, GSK-3$\beta$ may also be regulated by nutrient availability through the mTOR–S6k pathway (Peyrollier *et al.* 2000; Armstrong *et al.* 2001; Zhang *et al.* 2006). It is possible that lower growth factor and nutrient levels in the aged animals may have led to the constitutive phosphorylation of GSK-3$\beta$, irrespective of the stretch stimulus. Further experiments elucidating the mechanisms of GSK-3$\beta$ regulation in the vasculature would help to improve our understanding of alterations in load-induced vascular signalling with ageing.

Vascular smooth muscle is the least specialized type of muscle. Indeed, it is thought that vascular smooth muscle is phenotypically and morphologically more similar to non-muscle cells (e.g. fibroblasts) than striated or cardiac muscle in that smooth muscle does not have distinct myofibrils (Halayko & Solway, 2001). In addition, both smooth muscle and non-muscle cells respond to mechanical stimuli, such as tension or cyclic strain, with actin cytoskeletal rearrangement that is associated with increased contraction (Smith *et al.* 2000). Whether these characteristics alone or in combination with other factors are responsible for the differences in mechanically regulated signalling between smooth and striated muscle, reported by others, is unclear.

### Load-induced activation of the MAPK and p70 S6k pathways is affected differently with age

To our knowledge, this is the first report to examine the effects of age on uniaxial smooth muscle stretch. It is likely that the effects of ageing on mechanically regulated signalling transduction may differ with tissue type. For example, Hornberger and co-workers, using a uniaxial stretch stimulus, have reported that ageing does not affect the mechanically induced phosphorylation of the MAPK and p70 S6k pathways in skeletal muscle (Hornberger *et al.* 2005). Conversely, multiaxial stretch of the rat aorta appears to be associated with an age-associated decrease in the extent of load-induced phosphorylation of the p38- and Jnk-MAPK proteins (Rice *et al.* 2005*a*). In the present report, we fail to demonstrate any evidence of an age-associated attenuation in the ability of the aorta to activate MAPK signalling following a uniaxial stretch stimulus. The reason(s) underlying why ageing may alter the stretch-induced response to one type of stretch stimulus and not another is unknown. Since other stimuli (e.g. metabolic, growth factors) in addition to tension may be involved in MAPK activation, additional experiments will be required to address these possibilities.

Although MAPK signalling appears to be unaffected by ageing, we show that the ability of the aorta to increase Akt (Ser 473) with ageing is diminished. The exact mechanism of Akt (Ser 473) phosphorylation and the identity of kinase(s) responsible for the phosphorylation of this site have remained elusive. Recently, it has been hypothesized that integrin-linked kinase (ILK) may be involved (Kuemmerle, 2003); however, whether ILK regulates Akt (Ser 473) phosphorylation in VSMC is unknown. The ILK has been shown to be activated by mechanical stretch (Bradley *et al.* 2003) and is thought to play a role in regulating cytoskeleton reorganization in non-myocytes, such as fibroblasts and epithelial cells (Novak *et al.* 1998; Oloumi *et al.* 2004). Whether ILK performs a similar function in VSMC or whether the activity of ILK is altered with ageing awaits further clarification. The magnitude of PTEN dephosphorylation in response to uniaxial stretch decreased with age, suggesting that there may be a decreased inhibition of the $PI_3$ kinase–Akt pathway with age, further suggesting a heightened survival response.

## Conclusion

In conclusion, the present findings suggest that smooth muscle is similar to skeletal muscle in its ability to distinguish between stretch types. In smooth muscle, our data are consistent with the premise that the stretch-induced phosphorylation of MAPK proteins occurs independently of stretch type (uniaxial *versus* biaxial). Conversely, the activation of p70 S6k and its upstream activator, Akt, is sensitive to stretch type. Comparing across age groups, we show no change in the degree of axial stretch-induced MAPK phosphorylation while, conversely, the activation of Akt decreases. Taken together, these data suggest that different mechanically sensitive pathways exhibit different abilities to distinguish between stretch type and resistance to age-associated alteration.

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