

**ERECTILE DYSFUNCTION: AN EARLY MARKER FOR HYPERTENSION ?
A LONGITUDINAL STUDY IN SPONTANEOUSLY HYPERTENSIVE RATS**

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Running Head : Natural history of erectile dysfunction in SHR

Abstract

Erectile dysfunction (ED) is another manifestation of vascular disease. We evaluated the natural history of ED in the SHR and the respective participation of associated pathophysiological modifications i.e. endothelial dysfunction and tissue remodelling. SHR and their normotensive counterparts (WKY) of 6, 12 and 24 weeks of age (n=12) were used to evaluate erectile function, erectile and aortic tissue reactivity and remodeling. Erectile responses in SHR are reduced at all ages ($p < 0.001$). In both aortic and erectile tissues of SHR and WKY, relaxations to ACh are altered progressively with age, although more markedly in SHR. They are decreased at 12 weeks of age in erectile tissue of SHR compared to WKY (maximal relaxation: $-19.2 \pm 2.8\%$ vs -28.3 ± 3.9 , $p < 0.001$) but only at 24 weeks of age in aortas ($-47.9 \pm 6.4\%$ vs -90.5 ± 2.9 , $p < 0.001$). Relaxations to sodium nitroprusside are unaltered in aortic rings of both strains but enhanced in erectile tissue of SHR at 12 weeks of age. Major modifications in the distribution of collagen I, III and V in SHR occur in both type of tissue and are detectable sooner in erectile tissue compared to aortic tissue. The onset of ED is detectable prior to the onset of hypertension in the SHR. Structural and functional alterations, while similar, occur earlier in erectile compared to vascular tissue. If confirmed in humans, ED could be an early warning sign for hypertension and common therapeutic strategies targeting both ED and hypertension could be investigated.

Key words: hypertension, shr, endothelial dysfunction, remodeling, erectile dysfunction

Introduction

It is well-known that men with hypertension (HTN) have a significantly higher prevalence of erectile dysfunction (ED) than the general population (11; 16). Furthermore, the incidence of ED is associated with the duration and severity of HTN (11). Interestingly, 8-10% of untreated hypertensive patients suffer from ED at the time of diagnosis of HTN (19).

The basal tone of corpus cavernosum smooth muscle is controlled by complex events coordinated at the level of the central and peripheral nervous system. The sympathetic nervous system insures flaccidity by producing an α -adrenergic-dependent tone of the corporal smooth muscle maintaining the penis in a flaccid state, thus minimizing intracavernosal blood flow and pressure (ICP) (13). Upon sexual stimulation, penile erection, occurring in response to the activation of pro-erectile autonomic pathways, is greatly dependent on adequate inflow of blood to the erectile tissue and requires coordinated arterial endothelium-dependent vasodilatation and sinusoidal endothelium-dependent corporal smooth muscle relaxation (3). Nitric oxide (NO) is the principal peripheral pro-erectile neurotransmitter which is released by both non-adrenergic, non-cholinergic (NANC) neurons and the sinusoidal endothelium to relax corporal smooth muscle through the cGMP pathway (5; 15), and resulting ultimately in increased ICP (3). This increase in ICP activates pressure-dependent veno-occlusive mechanisms to limit the outflow of blood, thus further promoting elevated ICP and erectile response. The increased blood flow is thus ultimately driven by the force of the arterial pressure (13). Any factors modifying the basal corporal tone, the arterial inflow of blood to the corpora, the synthesis/release of neurogenic or endothelial NO within the corpora and/or the veno-occlusive mechanism are prime suspects for being involved in the pathophysiology of ED.

We have previously shown that erectile tissue from SHR with established hypertension presented functional alterations (6) mirroring those present in arteries from both conductance and resistance vasculature i.e. an elevated vasoconstrictor tone (29) and decreased endothelium-dependent vasodilatations (18). To go a step beyond, modifications of extracellular matrix composition (remodeling) at the vascular level are present, or may even precede onset of HTN (2; 12), thus modifying the overall dilatory capacity of the vessel (12). Interestingly, remodeling associated with hypertension may also occur at the corporal level (9; 14; 23; 26), although the timeline of these modifications is unknown at this time.

Therefore, the primary goal of this study was to study the natural history of ED associated with HTN *in vivo* in a well-established model of genetic HTN i.e. the spontaneously hypertensive rat (SHR) and its normotensive control rat, the WKY. Our second objective was to investigate the participation of the pathophysiological mechanisms implicated in ED in the SHR by studying in parallel the modifications occurring with time at the vascular level and at the level of the erectile tissue in terms of (1) tissue reactivity by *in vitro* isometric tension studies and (2) modifications in cellular and extracellular composition (remodeling).

Methods

Male SHR/Kyo@Rj male rats and WKY/Kyo@Rj rats at 6-, 12- and 24-weeks of age were obtained from Elevage Janvier (Le Genest-St-Isle, France). All procedures were performed in accordance with the *Guiding Principles in the Care and Use of Animals* established by the American Physiological Society (1) and the legislation on the use of laboratory animals (NIH publication N°85-23, revised 1996 and Animal care Regulations in force in France as of 1988).

In vivo evaluation of erectile function

As previously described (6; 13), SHR (n=12/age) and WKY (n=12/age) were anaesthetized with an intraperitoneal injection of xylazine (10mg/kg) and ketamine (90mg/kg), tracheotomized and maintained at 37°C. The carotid artery was catheterized to record arterial pressure (AP) and a 21-gauge needle was inserted into one of the corpus cavernosum (CC) of the penis to record intracavernosal pressure (ICP) simultaneously via pressure transducers (Elcomatic 750, UK). The cavernous nerve (CN) was exposed at the lateral aspect of the prostate and mounted on a bipolar platinum electrode connected to an electrical stimulator (AMS 2100, Phymep, France). For each animal, electrical stimulations of the CN (square-wave pulses of 1 ms, duration of 45 seconds, 6 V) at different frequencies (1, 2, 3, 4, 5 and 10 Hz) were performed in a randomized manner and repeated twice in view of establishing frequency-response curves. The erectile responses elicited by each electrical stimulation were quantified by calculating the ratio of $\Delta\text{ICP (mmHg)} / \text{MAP (mmHg)} \times 100$, with ΔICP being the difference between ICP in the flaccid state *i.e.* before stimulation and ICP during the plateau phase and MAP, the mean arterial pressure during the plateau phase. This ratio accounts for the strong influence of the systemic blood pressure in the amplitude of ICP

increase during the plateau phase (6; 13). At the end of the experiments, rats were sacrificed with an overdose of urethane.

In vitro isometric tension studies on aortic rings and strips of corpus cavernosum

Segments of thoracic aorta and strips of corpus cavernosum from the same anesthetized rat (pentobarbital, 50 mg/kg, ip, n=12/age/strain) were placed in 5-ml organ baths filled with Krebs physiological solution at 37°C bubbled with 95% O₂-5%CO₂ (6). Concentration-response curves to cumulative additions of ACh (10⁻⁸ to 10⁻⁵ or 10⁻⁴ mol/L, depending on the tissue) and sodium nitroprusside (SNP, 10⁻⁸ mol/L to 10⁻⁶ or 10⁻⁵ mol/L, depending on the tissue) were obtained in tissues precontracted with phenylephrine (10⁻⁵ M or 3x10⁻⁵ M for corpus cavernosum and 10⁻⁶ or 3x10⁻⁶ for aortic rings, depending on the individual responses of each tissue in order to maintain comparable levels of stimulated forces between SHR and WKY). Concentrations inducing 50% of the maximal effect were expressed as pD₂ values using GraphPad Prism.

Evaluation of vascular and penile remodeling

Erectile and aortic tissue samples (n=24/age/strain) similar to those used for *in vitro* experiments were homogenized in a glass potter at 4°C in PBS for collagen determination or Tris-HCl pH7 (10 mM) containing 0.34 M sucrose, 1 mM EDTA, 1 mM PMSF, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 10 µg/ml pepstatin and 2 mM DTT for α-actin determination.

Analysis of collagen content

Tissue samples were hydrolyzed in 6M HCl at 105°C for 24 h and then desiccated. The desiccated hydrolysate was submitted to a differential separation of proline and

hydroxyproline using a toluene fractionation procedure (17). Protein content was quantified in proline extract by colorimetric assay with ninhydrine. Collagen content was determined in hydroxyproline extract with Ehrlich's reagent (para-amino benzaldehyde). The amount of total protein and collagen was determined using the Wiestner formula (mg protein = mg proline x 5.4; mg collagen = mg hydroxyproline x 7.46) (28).

Extent of extracellular matrix solubilisation determined by differential 4°C/30°C pepsin treatment

Before collagen phenotyping, pepsin digestion (pepsin:collagen ratio of 1:5 in 0.5 M acetic acid) was performed at 4°C for 48 hours. After centrifugation, a second total digestion (same pepsin:collagen ratio at 30°C for 72 hours) was performed on the pellet. Quantity of collagen was determined on each pepsin extract by colorimetric assay of hydroxyproline.

Typing of collagen

Type I, III and V collagen in the pooled pepsin extracts were separated on 7.5% SDS-PAGE in reducing conditions (17). Collagen α -chains were stained by Coomassie blue, identified using standard collagen I, III and V samples, quantified in triplicate by densitometric analysis (Syngene GeneTools, UK) and collagen proportions were calculated as previously described (17): the percentage of collagen I equals $100 \times [\alpha 2(I)] \times 3 / \text{total alpha chains}$, % of collagen III = $100 \times [(\alpha 1(I) + \alpha 1(III)) - [\alpha 2(I)] \times 2] / \text{total alpha chains}$ and % collagen V: $100 \times [\alpha 1(V)] \times 1.5 / \text{total alpha chains}$.

Smooth muscle cell content (α -actin determination)

Total protein concentration was measured with a Bradford assay and equal amounts of protein (0.3 μ g) were separated on 10% denaturing SDS-PAGE, electroblotted on nitrocellulose, probed with a monoclonal anti- α -actin antibody (ABCAM, UK) and detected

by enhanced chemiluminescence (Roche, Germany) on autoradiographic film (Kodak Bio-Max Light film, USA). Densitometric results (Quantity one software, Biorad, USA) were expressed in relative optical density of the α -actin protein normalized by an internal standard. This internal standard was a fixed amount of an aortic sample run on the same gel than all other samples and following the same detection process than the samples with which it was run. Thus, normalization with this internal standard insured comparable results from one gel to another.

Values were expressed as mean \pm SEM. Comparisons were performed using one-way or two-way ANOVA with repeated measures followed by Bonferroni's complementary analysis where relevant. A p value less than 0.05 was considered to be significant.

Results

Mean arterial pressure was significantly higher in anaesthetized SHR of 12 and 24 weeks of age compared to WKY (113 ± 4 versus 96 ± 4 and 121 ± 4 versus 96 ± 4 , $p<0.001$, respectively) but not at 6 weeks of age (94 ± 4 versus 83 ± 2 mmHg, NS).

Evaluation of erectile function in vivo

Erectile responses to CN stimulation from WKY were identical at 6-, 12- and 24 weeks of age. Likewise, in SHR, there was no further worsening of erectile responses to CN stimulation with time, indicating that the ratio of ICP/MAP remained equivalent for a given frequency, thus that the rise in ICP varied proportionally to the rise in MAP in these SHR. Strikingly, however, the magnitude of the erectile responses was drastically reduced in SHR compared to WKY rats at all time points and all frequencies except 1 Hz ($p<0.001$, Figure 2).

Isometric tension studies on aortic rings and corporal strips

In both aortas and corporal strips, the initial tension generated in response to PHE (10^{-6} M or 10^{-5} M, respectively) was not modified with time in WKY while it was significantly decreased in 12- and 24-weeks-old SHR compared to younger 6-weeks-old SHR (In corporal strips: 206 ± 18 g/g ww and 177 ± 18 g/g ww vs 334 ± 43 g/g ww, and in aortic rings: 405 ± 60 g/g ww and 473 ± 76 g/g ww vs 741 ± 82 g/g ww, for 12- and 24-weeks-old SHR compared to younger 6-weeks-old SHR, respectively). These developed tensions were significantly decreased in aortic rings of 24-weeks-old SHR compared to age-matched WKY (405 ± 60 vs 756 ± 98 g/g ww) and as soon as 12 weeks of age in corporal strips (206 ± 18 vs 265 ± 18 g/g ww). In subsequent experiments, care was taken to obtain comparable precontraction

levels with PHE before inducing concentration-dependent relaxant responses to ACh or SNP in both WKY and SHR.

In aortic rings, maximal relaxation responses to ACh were reduced progressively with increasing age in SHR starting at 12 weeks of age while they were unchanged in WKY. Interestingly, the pD_2 values in 6-weeks old WKY indicate a particular sensitivity and responsiveness to ACh at this age (8.44 ± 0.27 versus 7.43 ± 0.20 and 7.60 ± 0.08 at 12 and 24 weeks of age, $p < 0.05$ and $p < 0.01$, respectively). Furthermore, at 24-weeks of age, the concentration-response curve was significantly different between WKY and SHR, indicating a clear alteration of the ACh-induced endothelium-dependent relaxations in aortic rings from SHR (Figure 3). Maximal relaxation to SNP and pD_2 values did not differ significantly among the different age groups in WKY or in SHR, except at 24 weeks of age (pD_2 values of 8.72 ± 0.12 in SHR versus 9.11 ± 0.09 in WKY, $p < 0.05$, Figure 4).

In corporal strips, in both WKY and SHR, maximal relaxation responses to ACh were clearly diminished at 12 and 24 weeks of age compared to 6 weeks of age indicating a particular sensitivity and responsiveness to ACh at that age. Furthermore, the concentration-response curves to ACh were significantly altered in SHR compared to WKY as soon as 12-weeks of age while pD_2 values were significantly augmented (7.02 ± 0.19 versus 6.46 ± 0.12 , $p < 0.05$, Figure 3). Maximal relaxation responses to SNP were slightly reduced with increasing age in WKY but not in SHR. The degree of relaxation elicited by SNP was even greater in SHR starting at 12 weeks of age, with greater pD_2 values compared to WKY (7.11 ± 0.08 versus 6.46 ± 0.12 , $p < 0.001$, Figure 4).

Vascular and penile remodeling

In both aortic and erectile tissues, there was a significant increase in total protein content with age in both strains, with WKY tissue containing more protein per gram of wet weight than age-matched SHR ($p < 0.05$, Table 1). In both strains, while total collagen remained constant with age in aortic rings, it increased significantly with age in the erectile tissue, with a more pronounced accumulation in SHR compared to WKY, especially at 24 weeks of age ($p < 0.05$, Table 1).

A 4°C-pepsin treatment solubilized more than 95% of total collagen in aortic tissue from both strains and more than 90% in erectile tissue from WKY whatever the age (Table 1). In contrast, the proportion of 4°C-pepsin soluble collagen decreased significantly with age in erectile tissue from SHR, and was significantly lower in SHR compared to WKY starting as early as 6 weeks of age (Table 1). An additional extraction step with pepsin at 30°C was thus performed to allow the solubilization of up to 90% of total collagen present in SHR erectile tissue and the two pepsin extracts were mixed for further analysis.

Both aortic and erectile tissue from WKY and SHR aorta contained type I, III and V collagens, with type I as the major collagen present. Most interestingly, the relative distribution of collagen subtypes was similar in erectile and aortic tissues although great differences remain between WKY and SHR in both type of tissues. Indeed, collagen I/III ratio increased with time in WKY aortas ($p < 0.05$) while it strongly decreased at 12 and 24 weeks of age in SHR aortas compared to WKY (Figure 5, right panel). In corpus cavernosum, the collagen I/III ratio is already significantly reduced in SHR erectile tissue at 6 weeks of age compared to age-matched WKY erectile tissue and continues to decrease thereafter. Collagen V accumulation was increased in SHR aortas compared to WKY at 24 weeks of

age, while it was detectable as soon as 12 weeks of age in the erectile tissue (Figure 5, left panel).

While there was no difference in the α -actin content of aortic and erectile tissue in SHR with time, there was a significant increase in α -actin content in 12-weeks-old WKY compared to 6 and 24 weeks-old WKY in both erectile and aortic tissues and in 12-weeks-old WKY compared to age-matched SHR in aortic tissues (Table 1, Figure 6). On the other hand, when comparing WKY and SHR at 6 and 24 weeks of age, there was no difference in aortic α -actin content while there was a significant increase in α -actin content in erectile tissue of SHR compared to WKY (Table 1, Figure 6).

Discussion

Because we and others had already evidenced that SHR with established HTN (6; 14) or other hypertensive rats (DOCA-salt and SP-SHR) (9) had ED compared to normotensive rats, the present study aimed to evaluate the natural history of corporal and vascular structural and functional abnormalities due to the progression and development of HTN and its consequences on erectile function in the SHR. We report that the magnitude of erectile responses is considerably reduced in the SHR compared to age-matched WKY, whatever the age, indicating that the onset of ED is detectable prior to the onset of HTN, without further deterioration with time. Interestingly, similar structural and functional alterations occur in the endothelium of vascular and corporal origin, but with a different time course. Indeed, our data demonstrate altered corporal endothelium-dependent relaxations occurring earlier than aortic alterations, as soon as HTN is established. Moreover, consistent parallel changes in the cellular and acellular tissue composition from SHR are also detectable at an earlier time-point in the erectile compared to the aortic tissue and characteristic of a fibrotic remodeling.

A finding of clinical relevance in these experiments is that, despite the fact that the magnitude of an erectile response is directly driven by the magnitude of the arterial blood pressure (13), erectile responses elicited by CN stimulation are already decreased in the pre-hypertensive SHR and do not evolve with time thereafter. For obvious reasons, the measurements could not be obtained in freely moving conscious rats and we have previously looked at the possibility of interference of anaesthetics on these erectile responses, thus selecting ketamine/xylazine anaesthesia as the agent of choice (6). Nonetheless, the development of hypertension in the SHR has already been well-described and the age of 6 weeks is regarded to be largely pre-hypertensive (24). If confirmed in humans, this finding could be of utmost interest since it could confer the valuable property for ED to be an early warning sign/sentinel

for HTN as it has been postulated for cardiovascular conditions in general. Supporting this suggestion is the fact that 8-10% of untreated hypertensive patients suffer from ED at the time of diagnosis of HTN (19).

We have also investigated the evolution with time of corporal endothelium-dependent and independent relaxations implicated in the local physiology of penile erection and compared it to modifications occurring at the aortic level. Although the important control site for pressure is at the level of the small arteries/arterioles, the parallel observations between corporal and aortic tissue is of value, since endothelium-dependent corporal relaxation mechanisms, as in the aorta, rely mainly on the release of biologically-active NO, while smaller vascular beds (i.e. resistance vessels) include the concomitant release of other endothelium-derived substances i.e. EDHF (4). Indeed, previous studies have suggested that the penile vasculature and the erectile tissue could undergo similar modifications to those occurring in the systemic vasculature (6; 14; 23; 29). In the present study, we show that both corporal and aortic endothelium-dependent relaxation responses to ACh are reduced progressively with increasing age in SHR compared to WKY, although the time course of that alteration is different between the two types of tissue. Indeed, corporal endothelium-dependent alterations occur at an earlier age than aortic alterations, as soon as HTN is established. This suggests that the erectile tissue of SHR is not protected from the functional changes induced by chronic exposure to high blood pressure, it could even be at the front line of the development of endothelial dysfunction, and thus be an early target end-organ.

Interestingly, we found that erectile tissue from both WKY and SHR possesses a particular sensitivity and responsiveness to ACh and SNP at 6 weeks of age and this particular reactivity is lost with time in both WKY and SHR, except for reactivity to SNP in the SHR. A

maturation of the calcium-sensitizing pathway involving RhoA-Rho kinase, the common downstream modulator of smooth muscle tone, could be involved, as already evidenced in vessels (21; 29). On the other hand, the increased sensitivity or effectiveness of SNP in corporal tissue from SHR at 12 and 24 weeks of age, is in agreement with previous results obtained in our laboratory (6) and could be a tissue-specific compensatory upregulation mechanism of the soluble guanylate cyclase pathway to defective endothelium-dependent relaxations (25). It is, however, not powerful enough to overcome other alterations present in the pre-hypertensive and hypertensive SHR and responsible for the altered erectile responses to sexual stimulation, suggesting that other mechanisms are involved. It is noteworthy, indeed, that impairment of the erectile responses to CN stimulation is already detectable in SHR as soon as 6 weeks of age while corporal and aortic endothelium-dependent alterations occur only once HTN is established. Thus, other mechanisms need to be addressed to understand ED in the pre-hypertensive SHR.

We have also evaluated the remodeling of the vascular and erectile tissues during the developmental stages of HTN in the SHR to evaluate its potential participation to ED. Indeed, it is well-recognized that vascular remodeling precedes the onset of HTN and participates in the long-term resistance changes associated with HTN (2; 27) and that structural changes in the penile vasculature also occur and may participate in the impairment of corporal smooth muscle relaxation leading to HTN-associated ED (14; 23; 26). Our data evidence the fact that striking and consistent changes in the distribution of collagen phenotypes in both erectile and aortic tissues from SHR compared to WKY occur. We clearly show greater percentage distribution of type V collagen and lower percentage distribution of collagen I/III in SHR, both in aortic and erectile tissues. These changes were detectable at an earlier time point in the erectile tissue compared to the aortic tissue (6 versus 12 and 12 versus 24 weeks of age,

respectively). These modifications in collagen distribution may be related to both the increase in smooth muscle mass we observed even in pre-hypertensive animals, at least at the level of the corpus cavernosum, and the predominance of a synthetic phenotype predisposing the vessels to the increase of extracellular matrix deposition. Indeed, the fact that these modifications occur both at the vascular and the corporal level points out to a common biosynthesis defect of extracellular matrix by the smooth muscle cells.

Interestingly, such collagen III overexpression has often been reported as the hallmark of the fibrosis-related alterations in several tissues during hypertension (8; 20). The relative augmentation of collagen III and V could contribute to the formation of less structured and less functional heterotypic collagen fibrils, as previously described (7). Thus, a general fibrotic process occurs during the development and evolution of HTN, as previously suggested (8; 20). Such alterations of collagen I/III and collagen V proportions in heterotypic fibrils of collagen, could lead to functional disturbances of the collagenous network within the erectile and aortic tissue from SHR and thus be implicated in the pathogenesis of ED in these animals. Furthermore, we found that the proportion of 4°C-pepsin soluble collagen decreased significantly with age in erectile tissue from SHR, and was significantly lower in SHR compared to WKY starting as early as 6 weeks of age. Although this preliminary observation needs further investigations, it could be an early indication of a progressive cross-linkage of collagen in erectile tissue of SHR, contributing to its resistance to digestion by pepsin treatment and resulting in the formation of a fibrotic and less functional tissue.

In summary, and to the best of our knowledge, this is the first and most exhaustive report performed both *in vivo* and *in vitro* to investigate the progression of corporal and vascular structural and functional abnormalities associated with development of HTN and its

consequences on erectile function in SHR. Important features of fibrotic alterations of the corporal tissue were detected in the pre-hypertensive SHR and concomitant with ED. These structural changes do not have an impact on the endothelium-dependent and –independent relaxations of aortic and erectile tissue in the pre-hypertensive SHR. Although this result may be surprising, inappropriate extracellular matrix composition of the corpora cavernosa, may nonetheless be responsible for altered mechanical properties of the erectile tissue leading to corporal veno-occlusive dysfunction. Indeed, although relaxations of the erectile tissue is preserved, it may have lost the characteristic compliance of the fibroelastic frame of the penis (22), resulting in an inability to expand the trabeculae against the tunica albuginea and compress the subtunical venules. The clinical consequences for such hemodynamic alterations are an excessive outflow of lacunar blood through the subtunical venules, which prevents adequate penile rigidity, leading to corporal veno-occlusive dysfunction and thus ED.

This study provides an exhaustive experimental support to investigate common therapeutic strategies targeting both ED and HTN i.e. inhibitors of the renin-angiotensin system (ACE inhibitors or AT₁ receptor antagonists); all the more since recent studies have suggested that erectile function of the SHR could be recovered following such antihypertensive therapy (10; 14). In particular, this modelization could be particularly useful to investigate innovative pharmacological strategies acting upon remodeling. Indeed, this pioneering target could both address the modifications occurring at the level of the general vasculature but also specifically at the level of an original target end-organ i.e. the penis.

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Figure 1: Representative tracings of original recording of intracavernous pressure when stimulating the cavernous nerve (6 V, 10 Hz, 1 ms, 45 s) SHR and in age-matched WKY rats at 6 (left), 12 (middle) and 24 (right) weeks of age. Mean arterial pressures in anaesthetized SHR and WKY at rest were 94 ± 4 versus 83 ± 2 mmHg, 113 ± 4 versus 96 ± 4 mmHg and 121 ± 4 versus 96 ± 4 mmHg at 6, 12 and 24 weeks of age respectively.

Figure 2: Effect of cavernous nerve stimulation at increasing stimulation frequencies on the intracavernosal pressure (ICP) of SHR versus WKY rats of various ages. Results are expressed as ratio of $\Delta\text{ICP} / \text{MAP}$ (%) with ΔICP being the difference between ICP in the flaccid state and ICP during the plateau phase of the erectile response and MAP, the mean arterial pressure during the tumescence phase (n=12/age/strain). $***p < 0.001$, Two-Way ANOVA SHR versus age-matched WKY.

Figure 3: Relaxations induced by increasing concentrations of ACh ($10^{-9}/10^{-5}$ or 10^{-4} mol/L, depending on the tissue) in aortic rings (right panel) and corporal strips (left panel) from WKY and SHR at 6 (upper panel), 12 (middle panel) and 24 (lower panel) weeks of age (n=12/age/strain). $§§§p < 0.001$, SHR versus WKY, Two-way ANOVA and $**p < 0.01$, $***p < 0.001$, Bonferroni's complementary analysis.

Figure 4: Relaxations induced by increasing concentrations of SNP ($10^{-10}/10^{-6}$ or 10^{-5} mol/L, depending on the tissue) in aortic rings (right panel) and corporal strips (left panel) from WKY and SHR at 6 (upper panel), 12 (middle panel) and 24 (lower panel) weeks of age (n=12/age/strain). $§§§p < 0.001$, SHR versus WKY, Two-way ANOVA and $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, Bonferroni's complementary analysis.

Figure 5: Evolution of collagen I/III ratio (upper panel) and collagen V (in mg/g protein, lower panel) in aortas (right panel) and erectile tissue (left panel) of WKY and SHR with time. Values are expressed as mean \pm SEM (n=12/age/strain). $§§§p < 0.001$, SHR versus WKY, Two-way ANOVA and $***p < 0.001$, Bonferroni's complementary analysis.

Figure 6: Representative immunoblots of α -actin in the corpus cavernosum (upper panel) and aorta (lower panel) of WKY and SHR at 6, 12 and 24 weeks of age. Equal amounts of protein were loaded on each lane.

Table 1 Vascular and penile remodeling in WKY and SHR with time

<i>Tissue</i>	Group	Protein (mg/g protein)	Collagen (mg/g protein)	Collagen extraction (%)	α -actin (DO)	
<i>AORTA</i>	WKY	6 weeks	232.5±8.3	437.5±4.9	>95	1.56±0.26 †
		12 weeks	250.9±5.3	443.7±5.3	>95	2.87±0.27
		24 weeks	265.0±8.6	450.1±5.2	>95	1.01±0.32 †
	SHR	6 weeks	214.1±1.3 § *	426.2±6.3	>95	1.84±0.12
		12 weeks	244.3±0.4 *	422.9±6.0	>95	1.81±0.20
		24 weeks	250.2±4.0 *	439.9±9.2	>95	1.29±0.14
<i>CORPUS CAVERNOSUM</i>	WKY	6 weeks	178.0±1.9 † ‡	261.1±7.8	93.4±1.3	0.69±0.14 †
		12 weeks	156.0±6.7	242.0±9.0	90.1±1.4	2.11±0.14
		24 weeks	189.0±7.9	315.1±5.9	91.9±1.2	1.13±0.34 †
	SHR	6 weeks	160.9±0.4 *	237.0±5.1 	70.0±1.4 ***	2.26±0.4 **
		12 weeks	159.8±2.0	260.0±6.7 	64.9±1.5 ***	2.08±0.21
		24 weeks	175.8±2.1	355.0±14.8 *	58.9±1.6 ***	2.73±0.33 **

Values are expressed as mean ± SEM (n=12/age/strain). *p<0.05, **p<0.01, ***p<0.001 versus age-matched WKY, Student's *t*-test. †p<0.05

versus WKY 12 weeks, ‡p<0.05 versus WKY 24 weeks, §p<0.05 versus SHR 12 weeks, ||p<0.05 versus SHR 24 weeks, One-Way ANOVA.

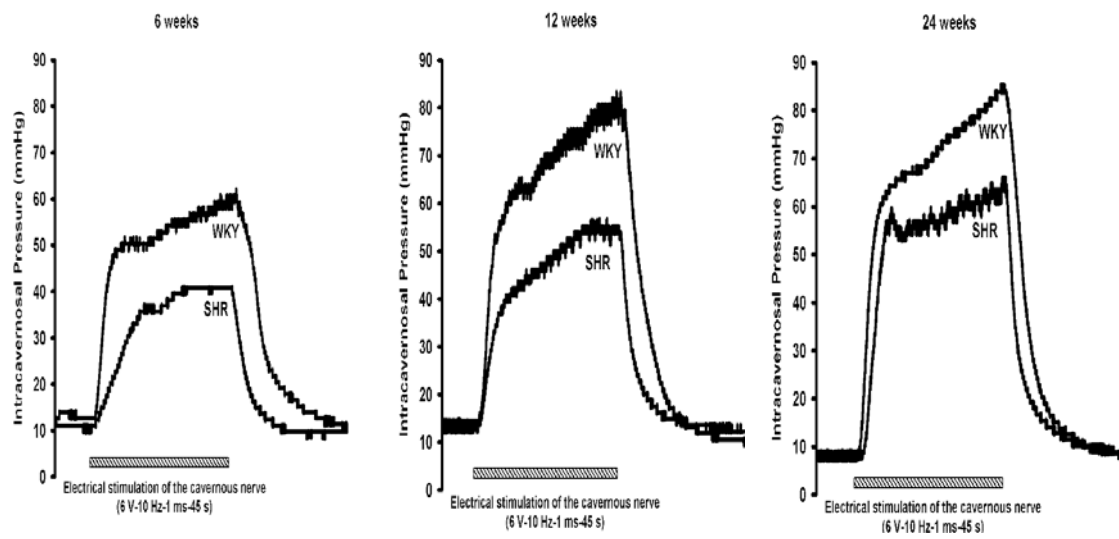


Figure 1

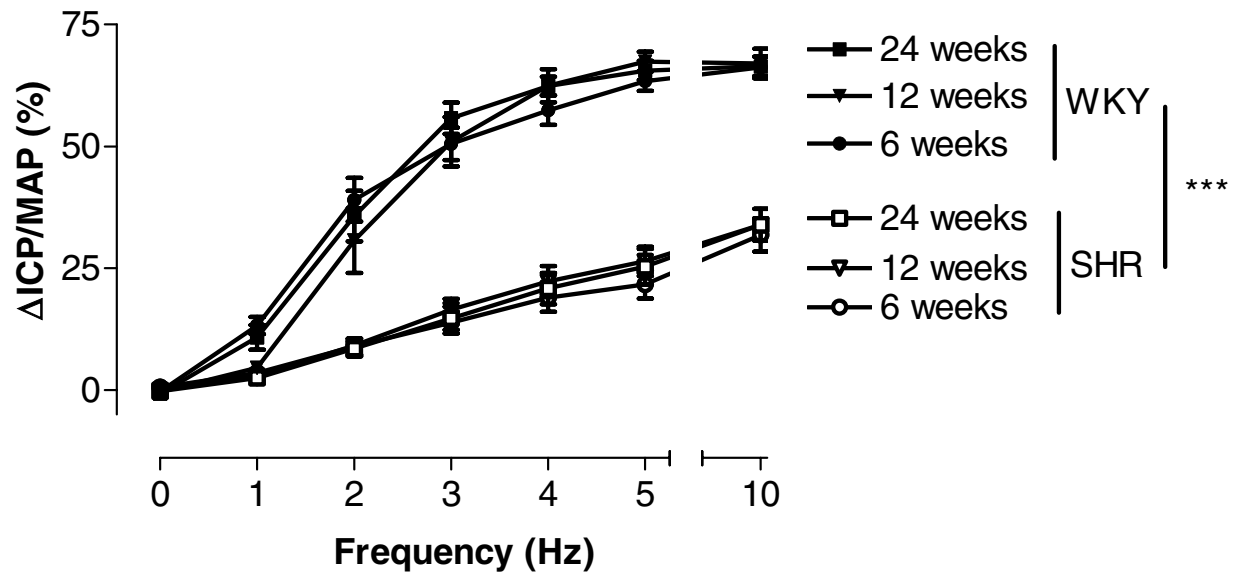


Figure 2

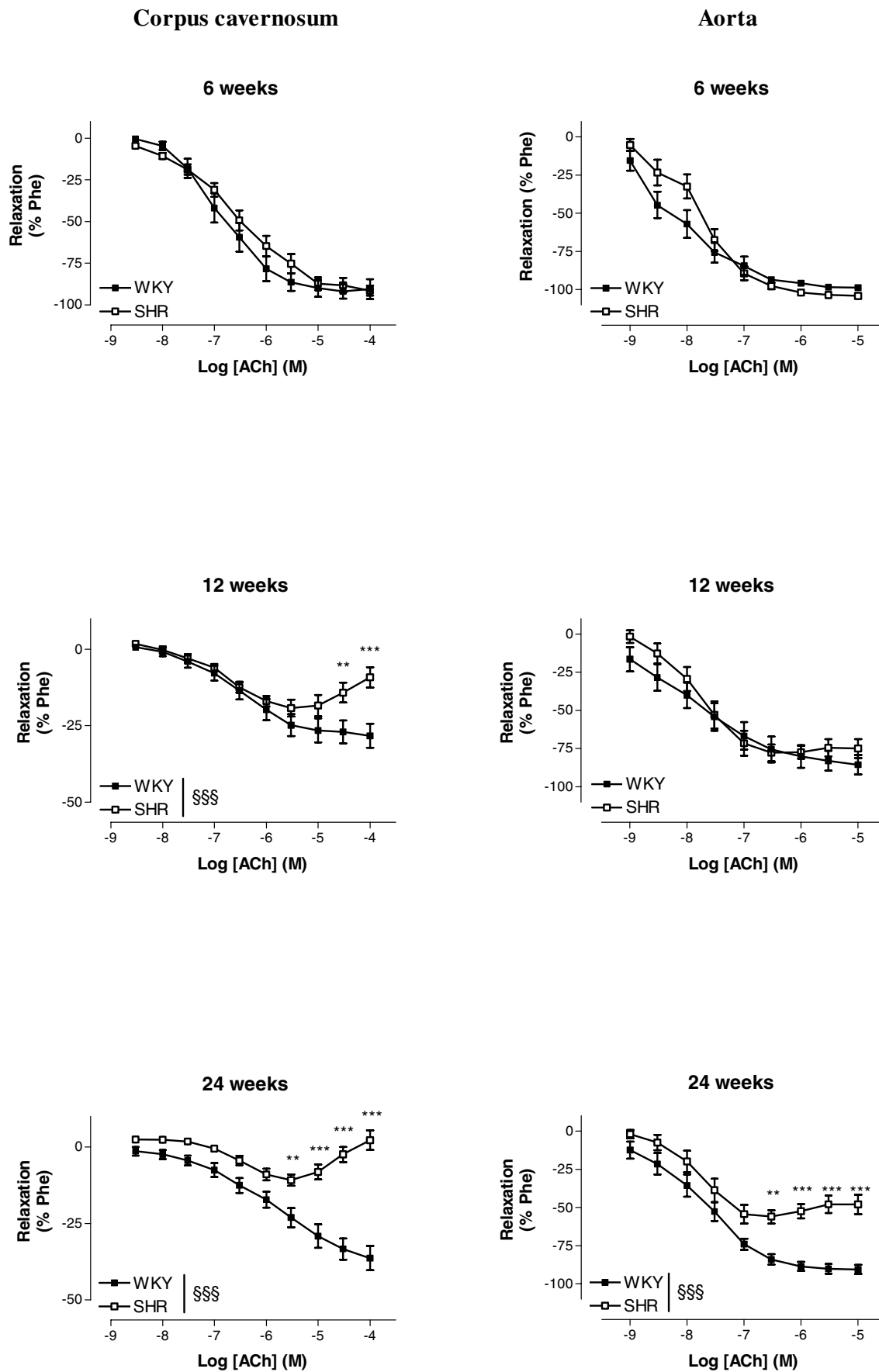


Figure 3

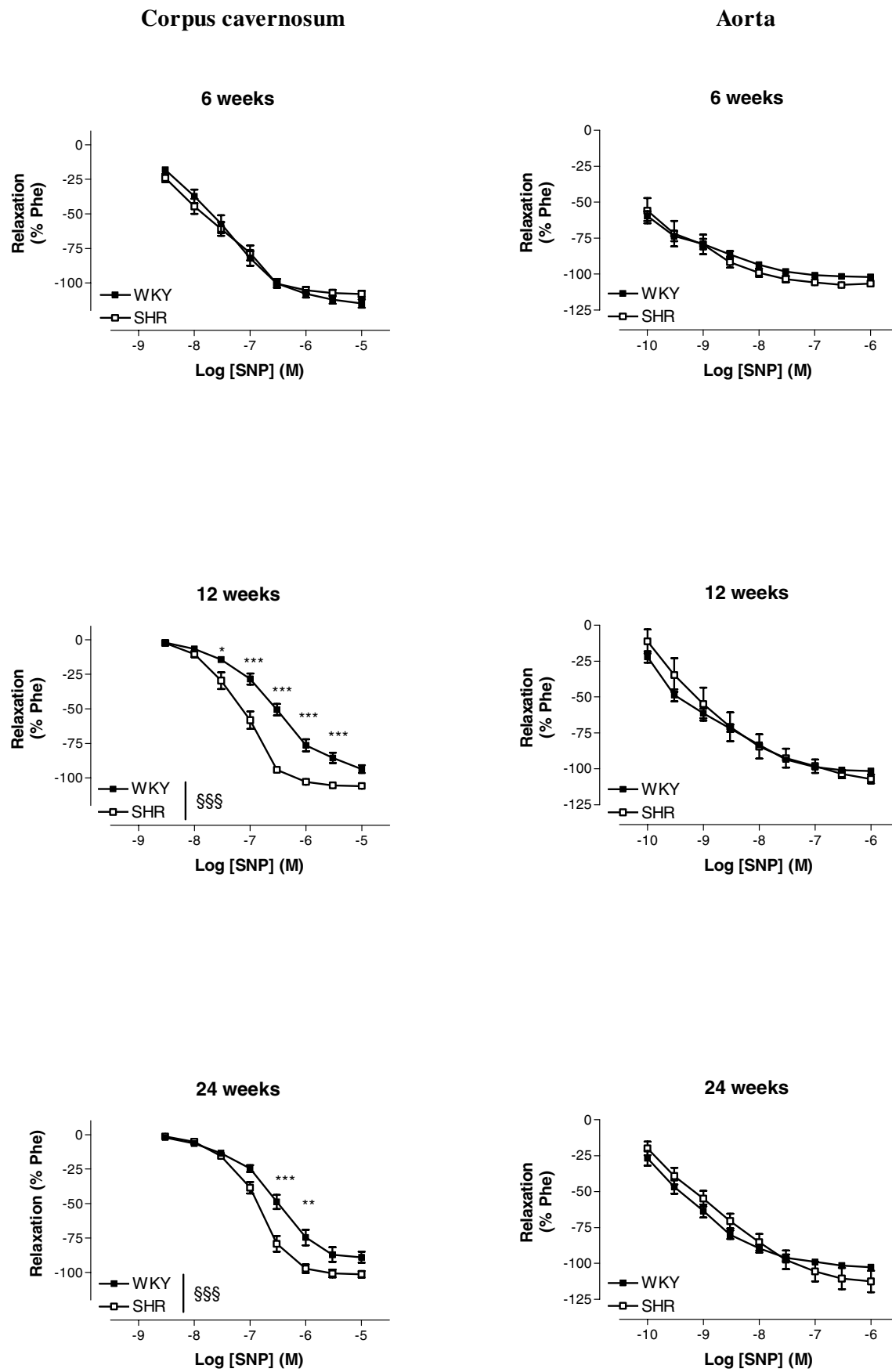


Figure 4

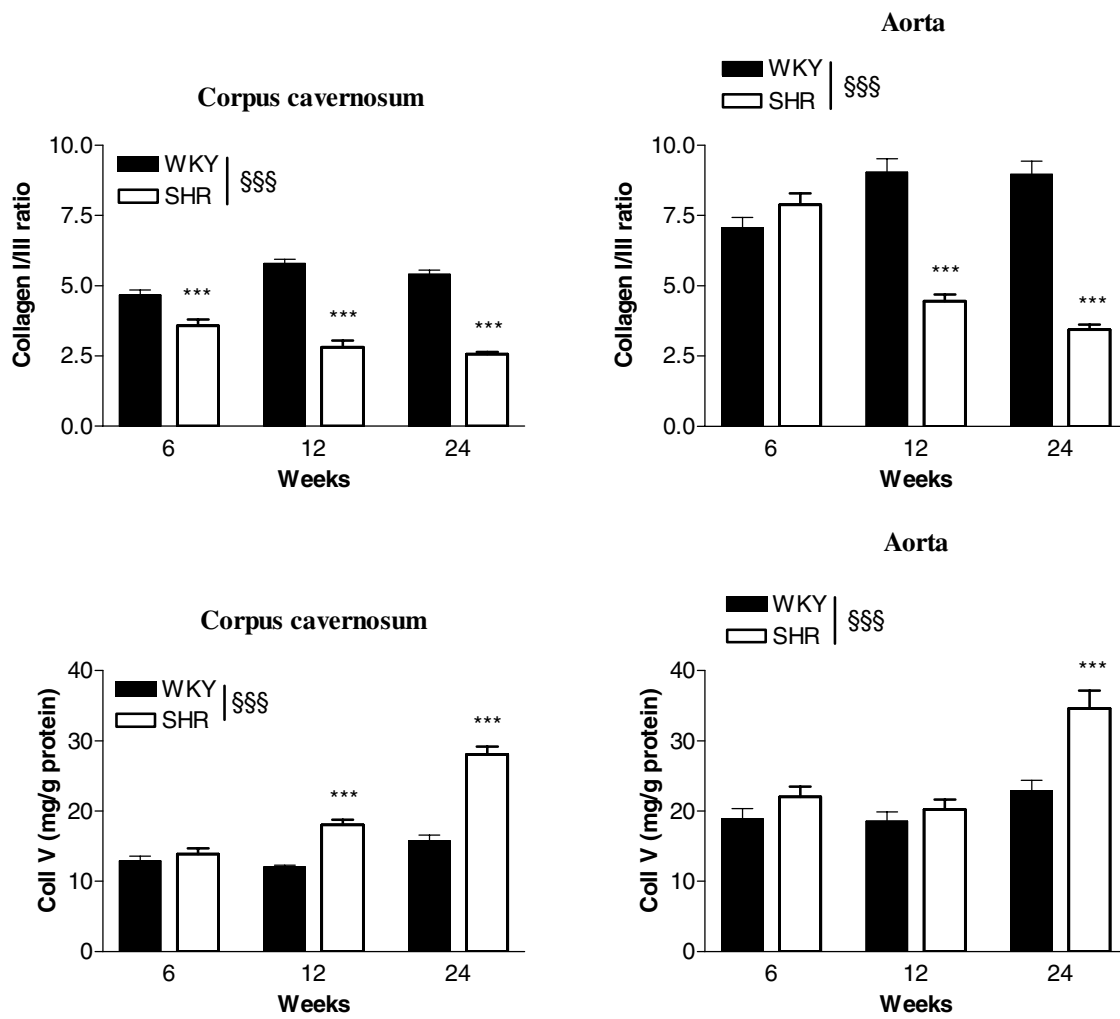


Figure 5

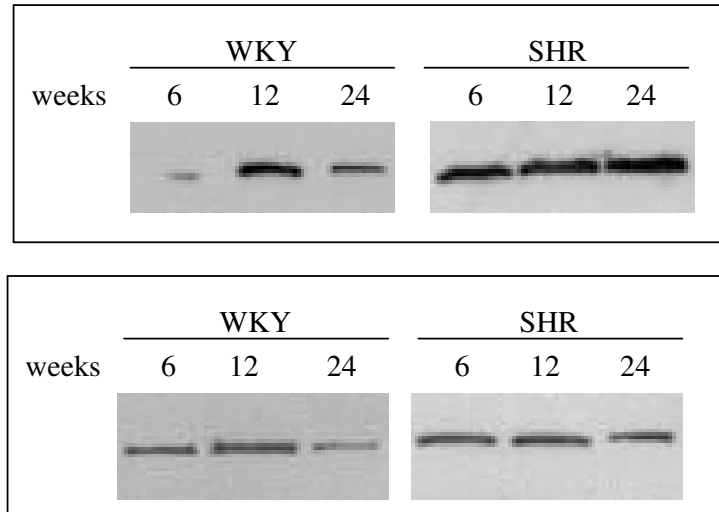


Figure 6