Smooth Muscle Cell-Specific Disruption of the BBSome Causes Vascular Dysfunction

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Abstract

The BBSome, a complex consisting of 8 Bardet-Biedl syndrome (BBS) proteins, is involved in the regulation of various cellular processes. Recently, the BBSome complex has emerged as an important regulator of cardiovascular function with implications for disease. In this study, we examined the role of the BBSome in vascular smooth muscle and its effects on the regulation of cardiovascular function. Smooth muscle-specific disruption of the BBSome through tamoxifen-inducible deletion of *Bbs1* gene, a critical component of the BBSome complex, reduces relaxation and enhances contractility of vascular rings and increases aortic stiffness independent of changes in arterial blood pressure. Mechanistically, we demonstrate that smooth muscle *Bbs1* gene deletion increases vascular angiotensinogen gene expression implicating the renin-angiotensin system (RAS) in these altered cardiovascular responses. Additionally, we report that smooth muscle-specific *Bbs1* knockout mice demonstrate enhanced endothelin-1 induced contractility of mesenteric arteries, an effect reversed by blockade of the angiotensin type 1 receptor with losartan. These findings highlight the importance of the smooth muscle BBSome in the control of vascular function and arterial stiffness through modulation of RAS signaling.

Summary

Smooth muscle-specific disruption of the BBSome causes vascular dysfunction and arterial stiffening. These vascular changes involve activation of the renin-angiotensin system as indicated by the increase in *angiotensinogen* gene expression and the ability of losartan to restores vascular function in mice lacking the BBSome in smooth muscle. Thus, the smooth muscle BBSome plays an important role in the regulation of vascular function and the development of arterial stiffness.

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Disclosures

The authors have nothing to disclose.

Keywords

BBSome; endothelial function; smooth muscle; renin-angiotensin system

Introduction

Despite considerable advances in understanding the underlying mechanisms and clinical treatment cardiovascular-related diseases remains a leading cause of mortality[1]. Vascular dysfunction and arterial stiffness are independent risk factors for the development of cardiovascular diseases including hypertension and stroke. Human and animal models of cardiovascular disease exhibit vascular dysfunction such as increased vascular contractility, decreased vasodilator function, and increased arterial stiffness[2–4]. Numerous underlying mechanisms of vascular dysfunction have been proposed such as signaling arising from the renin-angiotensin system (RAS), oxidative stress, and inflammatory pathways[5–7]. Indeed, altered RAS activity has been shown to be a major component in many cardiovascular diseases[8]. Additionally, blockade of the RAS has been demonstrated to restore vascular function in certain contexts[9–11] highlighting its importance in the regulation of vascular function and homeostasis. However, the mechanisms connecting RAS signaling and vascular dysfunction remain unclear and represent a critical gap in our understanding of cardiovascular-related diseases.

Bardet-Biedl syndrome (BBS) proteins have emerged as critical regulators of various cellular processes including gene expression and trafficking of receptors to cilia and the plasma membrane. Eight BBS proteins (BBS1, 2, 4, 5, 7, 8, 9 and 18) interact together to form a complex called the BBSome[12]. The BBSome is assembly through a process that involves another protein complex that contains 3 BBS proteins (BBS6, 10 and 12). On the other hand, BBS3 mediates the recruitment of the BBSome to the cell membrane whereas BBS17 controls the trafficking of this protein complex in cilia. The interconnection between the various BBS proteins explain the overlapping phenotypes that arise from mutations in any of the 21 *BBS* genes identified so far[13].

The high prevalence of hypertension and other cardiovascular risks in humans bearing mutations in *BBS* genes highlight the importance of these genes for cardiovascular regulation[14][15, 16]. Knockout mouse models of BBS recapitulate many of the phenotypes observed in BBS patients including hypertension supporting further the relevance of *BBS* genes for cardiovascular control[17–19]. Of note, variants in *BBS* genes have been associated with hypertension and other cardiovascular risks in non-BBS individuals[20].

We previously reported the expression of *BBS* genes in various cardiovascular-related tissues including the vasculature[18]. We showed that components of the BBSome, including *Bbs1*, are expressed in vascular cells (endothelial and smooth muscle) as well as in vessels such as the aorta[18]. We also demonstrated that mice bearing deletion of various *BBS* genes display contrasting changes in vascular functions[18]. For example, *Bbs6* null mice exhibit normal acetylcholine-induced relaxation responses compared to wildtype littermates whereas *Bbs2* null mice display enhanced acetylcholine-induced vasorelaxation despite both mouse models being obese. Presence of obesity is a confounding factor in using the global

null mice to dissect the role of the BBSome in the vasculature. In addition, the contribution of the BBSome in various cell types of the vasculature to the regulation of vascular function remains largely unknown.

In this study, we tested the hypothesis that the smooth muscle BBSome is required for the regulation of cardiovascular function. We generated a novel mouse model that allows temporal and selective disruption of the BBSome, through *Bbs1* gene deletion, in smooth muscle cells. We examined the effects of smooth muscle-specific *Bbs1* gene deletion on vascular function, blood pressure and arterial stiffening. Additionally, we provide evidence identifying the role of the RAS in the regulation of vascular function and arterial stiffening in response to smooth muscle BBSome disruption.

Methods

An expanded Methods section is available in the online-only Data Supplement.

The data that support the findings of this study are available from the corresponding author on reasonable request.

Animals

To generate a transgenic mouse line with smooth muscle-specific disruption of the BBSome, we crossed mice expressing a tamoxifen inducible smooth muscle-specific myosin heavy chain Cre promoter (Strain: B6.FVB-Tg(Myh11-cre/ERT2)1Soff/J; Jackson Labs #019079) with mice harboring floxed alleles of the *Bbs1* gene[21] from our colony. We then crossed these mice to a tdTomato reporter line (Strain: ROSA[Stop^F/F-tdTomato]) from our colony for visualization of Cre dependent tdTomato expression. The mice were maintained on a mixed background for this study (129SvEV/C57Bl/6J) as this breeding paradigm has been validated in our laboratory and represents consistency with previous studies[22][23]. All mice used in this study were males as the bacterial artificial chromosome transgene that encodes the inducible smooth muscle Cre recombinase (smMHC^CreERT2) was inserted on the Y chromosome. Cre-dependent recombination was induced via Tamoxifen injection (IP; 75mg/kg) in corn oil, which was used as vehicle, for 5 consecutive days at ~8 weeks of age. Molecular and physiological analyses were performed ~4 weeks after tamoxifen injections. We found no differences between mice with genotypes: SM^Cre/*Bbs1*^wt/wt + Tx and SM^Cre/*Bbs1*^F/F + Corn Oil (vehicle) for vascular function assays and refer to both groups as wildtype controls throughout the studies. All studies were approved by the University of Iowa Animal Research Committee.

Vascular function

Vascular function was assessed in aortic and mesenteric arterial rings from SM^CreERT2/*Bbs1*^F/F and wildtype mice. In brief, the thoracic aorta (3mm length rings) and second-order mesenteric arteries (2mm length rings) were dissected and cleaned of debris before they were mounted on a wire myograph (DMT; Model 610M). Aortic rings were mounted on steel pins and mesenteric arteries were mounted on 2 tungsten wires (25μm diameter) and placed in a 95%O_2/5%CO_2 Krebs solution containing the following (in mM): 118.3 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 2.5 $CaCl_2$, 11 Glucose, pH 7.4, 37C.

Resting tension was set at 0.5g for aortic rings and IC_{90} was applied to all mesenteric rings as previously described[18, 24] for 45 minutes. Aortic and mesenteric arterial rings were sub-maximally contracted with $PGF_{2\alpha}$ (aortic) or U-46619 (mesenteric) based on established protocols in our laboratory[25] and others[26] where smooth and sustained contraction was best achieved with these agonists, respectively. Vascular rings were then subjected to cumulative concentration response curves to acetylcholine (1nM-10μM) and sodium nitroprusside (1nM-10μM). Additionally, cumulative contractile responses to phenylephrine (1nM-100μM), $PGF_{2\alpha}$ (1μM-100μM), U-46619 (1nM-10μM), serotonin (1nM-100μM), endothelin-1 (0.01nM-100nM) and KCl depolarization (100mM) were also assessed. A subset of arteries was pre-incubated with losartan (1μM; 30min) prior to contractile or relaxation function assessment. Data are reported as percentage relaxation, maximum contractility (g), or maximum tension (mN/mm) generated. All vascular reagents were purchased from Sigma except for U-46619 (Cayman Chemical).

Pulse wave velocity

Pulse wave velocity (PWV) measurements were performed using Doppler ultrasound (MouseDoppler, Indus Instruments, TX). Mice were anesthetized with isoflurane (2%) and ECG recorded for heart beat analysis. Ultrasound waveforms were collected using 20 MHz ultrasound probes placed on the descending aorta and abdominal aorta near the iliac bifurication. Waveforms were collected over 5–8 cardiac cycles and the distance was measured between the ultrasound probes. Pulse wave velocities were calculated dividing the distance (meters) by time (seconds).

Statistical Analysis

All data are expressed as means ± SEM. Data were analyzed and graphed using GraphPad 7 Prism software. Group comparisons were made via unpaired t-test. Blood pressure and vascular reactivity assays were analyzed using a Two-way ANOVA with or without repeated measures and a Bonferroni's (2 groups) or Tukey's (3 or more groups) post hoc test. Differences are considered statistically significant if p<0.05.

Results

Validation of smooth muscle-specific *Bbs1* gene deletion

Consistent with our previous report[18], we found that the *Bbs1* gene is expressed in the smooth muscle cells isolated from the aorta (Figure S1 in the online-only Data Supplement). We also demonstrate the efficacy of using the $SM^{CreERT2}$ promoter to delete *Bbs1* in smooth muscle cells. Isolated primary aortic smooth muscle cells from $SM^{CreERT2}$/*Bbs1*$^{F/F}$ mice were infected with an adenoviral Cre construct (Ad-Cre) to induce recombination of LoxP sites. Ad-Cre infection reduced *Bbs1* gene expression by ~95% compared to Ad-GFP infected control smooth muscle cells (p<0.0001 via unpaired t-test; Figure S1).

Next, we generated mice lacking the *Bbs1* gene specifically in smooth muscle cells by treating the $SM^{CreERT2}$/*Bbs1*$^{F/F}$ transgenic mice with tamoxifen. We validated this mouse model by visualizing Cre dependent tdTomato expression in the vascular smooth muscle layer of tamoxifen treated $SM^{CreERT2}$/*Bbs1*$^{F/F}$/STOP$^{F/F}$-tdTomato mice. Of note, while

tdTomato (red fluorescence) expression was found in smooth muscle cells of the aorta, endothelial cells (stained with von Willebrand Factor; green) did not express tdTomato (Figure 1A). To further validate the model, we isolated aortas from wildtype and SM^{CreERT2}/*Bbs1*$^{F/F}$ mice and measured *Bbs1* gene expression by quantitative real-time PCR in whole aortic lysates. SM^{CreERT2}/*Bbs1*$^{F/F}$ mice demonstrated significantly reduced *Bbs1* transcript levels compared to wildtype controls ($p<0.05$ via unpaired t-test; Figure 1B).

We have previously demonstrated that tissue-specific disruption of the *Bbs1* gene can cause body weight effects such as obesity[23]. Thus, we sought to determine if smooth muscle deletion of the *Bbs1* gene had any body weight effects in mice. Compared to wildtype controls, SM^{CreERT2}/*Bbs1*$^{F/F}$ mice exhibited no change in body weight or weight gain after tamoxifen administration (Figure 1C–D).

Smooth muscle-specific *Bbs1* gene deletion alters vascular relaxation responses in the aorta but not the mesenteric artery

We next assessed the role of smooth muscle deletion of the *Bbs1* gene in the regulation of vascular relaxation in the aorta and mesenteric artery. Aortic rings of SM^{CreERT2}/*Bbs1*$^{F/F}$ mice displayed decreased endothelial-mediated vasorelaxation responses to acetylcholine compared to wildtype control mice (Figure 2A; $p_{interaction}<0.05$ via 2-way ANOVA with repeated measures). Endothelial-independent vasorelaxation responses to sodium nitroprusside (SNP) was also decreased in SM^{CreERT2}/*Bbs1*$^{F/F}$ mice relative to wildtype controls (Figure 2B; $p_{interaction}<0.05$ via 2-way ANOVA with repeated measures). The reduced acetylcholine and SNP responses of the aorta suggest alterations in nitric oxide (NO) signaling. However, expression ratio of phospho-eNOS (Ser1177) to total eNOS protein was unchanged in the aorta of SM^{CreERT2}/*Bbs1*$^{F/F}$ mice (Figure 2C).

Next, we assessed the role of smooth muscle-specific deletion of the *Bbs1* gene on mesenteric artery function, a more prototypical resistance vessel involved in the regulation of arterial pressure. We found that both endothelial-dependent acetylcholine and endothelial-independent SNP relaxation responses were intact in SM^{CreERT2}/*Bbs1*$^{F/F}$ mice compared to wildtype controls (Figure 2D–E) diverging from our findings in the large conduit aorta. These data suggest that *Bbs1* gene deletion in smooth muscle cells interferes with both endothelial and smooth muscle-mediated vasorelaxation responses in the aorta. SM^{CreERT2}/*Bbs1*$^{F/F}$ mice treated with corn oil (vehicle) displayed no differences relative to tamoxifen treated wildtype controls for relaxation responses of the aorta and mesenteric artery (Figure S2A–D) indicating the specificity of the SM^{CreERT2} driver.

Smooth muscle-specific *Bbs1* gene deletion alters contractile responses in the aorta and mesenteric artery

We next assessed whether smooth muscle-specific *Bbs1* gene deletion affects the ability of the aorta and mesenteric artery to contract in response to activating calcium. Maximal depolarization to KCl (100mM) was significantly elevated in SM^{CreERT2}/*Bbs1*$^{F/F}$ mice compared to wildtype controls in both the aorta (Figure 3A) and mesenteric artery ($p<0.05$ via unpaired t-test, Figure 3D). However, maximal aortic responses to prostaglandin (PGF$_{2\alpha}$) and phenylephrine (PE) were not different between SM^{CreERT2}/*Bbs1*$^{F/F}$ and

wildtype mice (Figure 3B–C). Strikingly, the mesenteric artery of SM^{CreERT2}/$Bbs1^{F/F}$ mice displayed increased agonist induced contraction to thromboxane (U-46619) and PE ($p_{interaction}$<0.05 via 2-way ANOVA with repeated measures, Figure 3E–F). No difference was detected in the contractile response evoked by serotonin (5-HT) in the aorta and the mesenteric artery of SM^{CreERT2}/$Bbs1^{F/F}$ mice (Figure S3). Interestingly, a significant increase in contractility of both the aorta and mesenteric artery for endothelin-1 (ET-1) was noted in SM^{CreERT2}/$Bbs1^{F/F}$ mice (Figure 4A–B; $p_{interaction}$<0.05 via 2-way ANOVA with repeated measures). These data indicate selectivity and vascular bed specificity in altered contractile responses after *Bbs1* gene deletion in smooth muscle cells. It should be noted that aorta and mesenteric artery contractile function were not altered in mice receiving vehicle (corn oil) injections (Figure S4A–J).

Smooth muscle-specific *Bbs1* gene knockout does not alter blood pressure but increases arterial stiffening of the aorta

We measured mean arterial pressure by radiotelemetry and found no differences between SM^{CreERT2}/$Bbs1^{F/F}$ and wildtype mice at both 2- and 4-weeks post-Tamoxifen administration (Figure 5A). Additionally, there was no differences between wildtype and SM^{CreERT2}/$Bbs1^{F/F}$ mice for systolic, diastolic or pulse pressure (Figure S5A–C) as well as heart rate or motor activity (Figure S5D–E). Interestingly, we found a significant increase in aortic pulse wave velocity measured 4 weeks post-Tamoxifen ($p<0.05$ via unpaired t-test; Figure 5B) indicating smooth muscle-specific *Bbs1* gene deletion causes aortic stiffening.

Smooth muscle-specific *Bbs1* gene deletion increases vascular angiotensinogen gene expression

The reduced acetylcholine and SNP responses paired with increased arterial stiffening in the aortas from SM^{CreERT2}/$Bbs1^{F/F}$ mice compared to wildtype controls suggests alterations in the RAS. Indeed, we found increased mRNA levels for angiotensinogen in the aorta (Figure 6A; $p<0.05$ via unpaired t-test) and mesenteric artery (Figure 6B; $p<0.05$ via unpaired t-test) of SM^{CreERT2}/$Bbs1^{F/F}$ mice compared to wildtype controls. No differences were observed in the expression of renin, ACE, angiotensin type 1a or 1b receptor, or the angiotensin type 2 receptor in the aorta (Figure S6A–E).

We also examined the expression of a variety of other genes involved in the regulation of vascular function. However, there were no significant changes in aortic expression of IL-1β, V-CAM1, SOD2, NOX1, NOX2 or NOX4 mRNAs in SM^{CreERT2}/$Bbs1^{F/F}$ mice compared to wildtype controls (Figure S7A–F). Similarly, we found no differences in protein expression and the activities of eNOS, Akt, ERK, and PKCα signaling in aortas of SM^{CreERT2}/$Bbs1^{F/F}$ mice compared to wildtype controls (Figure S8A–D).

Cross-talk between the RAS and ET-1 has been previously implicated in the regulation of contractile function[27, 28]. Thus, we tested whether activation of vascular RAS in SM^{CreERT2}/$Bbs1^{F/F}$ mice contributes to the increased ET-1 constriction of the aortic and mesenteric arterial rings. Consistent with such possibility, pre-incubating aortic rings with losartan (1μM; 30min) abolished the contractile responses to ET-1 in both SM^{CreERT2}/$Bbs1^{F/F}$ and wildtype mice (Figure 6C; $p_{interaction}$<0.0001 via 2-way ANOVA with repeated measures).

Interestingly, in the mesenteric artery pre-incubation with losartan reduced ET-1 contraction in SM[CreERT2]/*Bbs1*[F/F] mice without changing contractile response in wildtype mice (Figure 6D; $p_{interaction} < 0.0001$ via 2-way ANOVA with repeated measures). Restoration of ET-1 contractile function by losartan led us to test whether losartan treatment could also restore endothelial-mediated relaxation responses in aortic rings of SM[CreERT2]/*Bbs1*[F/F] mice. Indeed, pre-incubation of aortic rings with losartan restored acetylcholine-mediated relaxation responses in SM[CreERT2]/*Bbs1*[F/F] mice to the level of wildtype mice (Figure 6E; $p_{interaction} < 0.0001$ via 2-way ANOVA with repeated measures).

Discussion

In this study, we identified an important role for the smooth muscle BBSome in the regulation of vascular function and the development of arterial stiffness. Smooth muscle-specific deletion of the *Bbs1* gene, a critical component of the BBSome complex, results in impaired endothelial–dependent and –independent vascular relaxation responses, enhances contractile function, and increases arterial stiffening independent of changes in arterial pressure or body weight. Mechanistically, we demonstrate that smooth muscle *Bbs1* gene deletion lead to an increase in vascular angiotensinogen mRNA in two different vascular beds. Furthermore, blockade of the angiotensin type 1 receptor with losartan restores normal contractile function to endothelin-1 of smooth muscle-specific *Bbs1* gene null mice implicating the RAS in mediating the enhanced contractile responses evoked by smooth muscle BBSome disruption. These results highlight a complex and highly integrated pathway connecting the smooth muscle BBSome, the RAS, and cardiovascular function. Taken together, these data suggest that the smooth muscle BBSome is a novel regulator of vascular function and arterial stiffening.

It is well documented that vascular dysfunction is an important contributor to the development and progression of hypertension and that mitigation of these vascular deficits reduces cardiovascular risks[4]. To our knowledge, clinical measurements of vessel function in BBS patients have not been performed. However, using mouse models of BBS our laboratory has previously demonstrated contrasting changes in vascular relaxation and contractile responses depending on which *BBS* gene was globally deleted. Additionally, we have demonstrated expression in endothelial and smooth muscle cells as well as vascular tissues of several *BBS* genes including the *Bbs1* gene[18]. In BBS patients, the *Bbs1* gene is the most commonly mutated gene with a single missense mutation in methionine to arginine (M390R) accounting for ~80% of mutations in this gene[29]. We and others have previously demonstrated that deletion of the *Bbs1* gene recapitulates many of the clinical features of BBS.[21, 23]

To understand the contribution of the BBSome to the regulation of cardiovascular function, we developed a tamoxifen-inducible smooth muscle-specific *Bbs1* gene deletion mouse model. Our data demonstrate both impaired endothelial-dependent and -independent relaxation responses in aortic rings from smooth muscle-specific *Bbs1* knockout mice measured at 4 weeks post-Tamoxifen administration. Interestingly, these findings are unique to the aorta as we found no differences in relaxation responses in mesenteric arterial rings

suggesting both regional specificity and predominance of other vasodilator signals (EDHF or prostacyclin) for these responses depending on the vascular bed studied.

The activity and bioavailability of NO is a primary determinant of vascular relaxation particularly in large conduit vessels such as the aorta[4]. In this study, we found no changes in the expression of phospho-eNOS (Ser117) or total eNOS protein in aortic rings lacking the *Bbs1* gene in smooth muscle cells. NO levels can be modulated through the production of reactive oxygen species and proinflammatory cytokines[4]. We found no differences in mRNA expression for a variety of ROS-producing/degrading and inflammatory genes in smooth muscle *Bbs1* gene deleted mice compared to wildtype controls. These findings suggest that aortic endothelial relaxation function may be intact while the smooth muscle is unresponsive to the relaxing effects of NO in smooth muscle *Bbs1* gene deleted mice. We did not measure the expression of guanylate cyclase, cGMP levels, or eNOS uncoupling in this study. Additionally, we found no differences in expression of several ROS-related genes in the vasculature of smooth muscle *Bbs1* gene deleted mice. However, this was not an exhaustive investigation and the overall contribution of ROS signaling to the observed phenotypes cannot be excluded. Therefore, additional studies are needed to elucidate the role of smooth muscle BBSome disruption on impaired vascular relaxation responses and the signaling pathways involved.

Enhanced agonist-induced contractility contributes to and predicts the development of hypertension in a variety of human and animal models[30–32]. In this study, we found differentially enhanced contractile responses in the aorta and mesenteric artery to both receptor–dependent and –independent activation of the smooth muscle contractile apparatus indicating a pro-contractile vascular state. Specifically, the aorta of smooth muscle Bbs1 null mice displayed enhanced contraction to membrane-induced depolarization with KCl, but not to agonist-induced contraction. The reason for this is unclear but may be due to differential triggering thresholds to activating calcium by these signaling pathways or inhibition of myosin phosphatase in response to smooth muscle *Bbs1* gene deletion. Interestingly, resistance arteries of the mesenteric circulation demonstrated increased contraction to all agonists studied except for serotonin (5-HT). This finding of no contractile effect of 5-HT in aortic and mesenteric vascular rings is consistent with our finding of no changes in expression and activity of the MAP kinase signaling pathway. Contraction to endothelin-1 was significantly elevated in both aortic and mesenteric arterial rings of smooth muscle *Bbs1* gene deleted mice compared to wildtype. This finding is intriguing as endothelin-1 has been implicated in hypertension in both human[33, 34] and animal models[35]. Indeed, blockade of endothelin-1 receptor signaling improves vascular function in several models of hypertension[36–38]. This also translates to human hypertension as blockade of endothelin-1 receptors have been shown to improve acetylcholine-induced vasodilation in hypertensive humans[33].

We previously demonstrated that global deletion of individual genes that encode the BBSome subunits (*Bbs2* or *Bbs4*), a subunit of the BBS chaperonin complex that assembles the BBSome (*Bbs6*) or a component involved in the transport and localization of the BBSome (*Bbs3*), all result in obesity. Interestingly, *Bbs4*, *Bbs6* and *Bbs3* null mice exhibit elevated blood pressure while *Bbs2* null mice display normal blood pressure[17, 39]. These

findings are supported by human studies where variants of *Bbs4* and *Bbs6* but not *Bbs2* were associated increased risk of for the development of hypertension in the general population[20, 40] highlighting a complex connection between BBS proteins and regulation of blood pressure. We have previously reported that a global *Bbs1*[M390R] knock-in mouse model of BBS demonstrates several characteristics of BBS including obesity[41]. Interestingly, *Bbs1*[M390R] mice exhibit normal arterial pressure despite obesity[41]. In our current study, smooth muscle-specific deletion of the *Bbs1* gene did not affect body weight or arterial pressure at 4 weeks post-tamoxifen administration confirming the normal arterial pressure phenotype in the *Bbs1*[M390R] model. However, it remains to be determined if long-term chronic smooth muscle-specific BBSome disruption affect adiposity and blood pressure.

Arterial stiffening is a well-known predictor of cardiovascular disease[3] and has been demonstrated to precede the development of hypertension in human and animal studies[2, 42]. Of note, arterial stiffness displays regional variability depending on which vascular bed likely due to the timing and local gene environment within the specific vascular bed[43]. Using Doppler ultrasound pulse wave velocity, we demonstrate increased aortic stiffness in smooth muscle *Bbs1* deletion mice. This finding is in concert with the reduced relaxation responses of the aortic rings measured *ex vivo* suggests a direct effect of BBSome disruption in smooth muscle on arterial compliance. We did not measure collagen or elastin deposition in the extracellular matrix (ECM) of smooth muscle *Bbs1* gene deleted mice. Thus, further studies are warranted to elucidate the composition of the ECM and its contribution to the increased arterial stiffness in this model.

Aberrant activity of the RAS has been associated with a variety of cardiovascular diseases such as obesity and hypertension[44]. Numerous studies have previously demonstrated a direct relationship between RAS activation and abnormal vascular function in various contexts[11, 45, 46]. RAS-induced vascular impairment is primarily mediated through the angiotensin type 1 receptor (AT1) as administration of losartan, a selective inhibitor of AT1 receptors, improves vascular reactivity in a variety of cardiovascular settings[28, 47–49]. Our observation of impaired vascular endothelial and smooth muscle mediated relaxation responses as well as enhanced contractility to different contractile agonists led us to investigate the RAS as a potential mediator of these responses. Indeed, our data demonstrate elevated mRNA levels of angiotensinogen in vascular segments spanning large and small arteries (aortic and mesenteric). Interestingly, we found no changes in expression of other RAS genes such as RAS enzymes (renin or ACE) or RAS receptors (AT1 or AT2). We did not measure local vascular RAS activity or circulating angiotensin peptides *in vivo* in response to smooth muscle *Bbs1* gene deletion. However, consistent with the concept of increased local vascular RAS activity we found that blockade of the angiotensin type 1 receptor with losartan incubation of mesenteric arteries restored normal contractile function to endothelin-1 from smooth muscle *Bbs1* gene deleted mice. Furthermore, losartan incubation of aortic rings restored acetylcholine-mediated endothelial function demonstrating the protective effect of RAS blockade against smooth muscle BBSome disruption. These data suggest that elevated RAS in response to smooth muscle *Bbs1* gene deletion is an important regulatory pathway in the control of vascular function and may provide a targeting point for the cardiovascular disease associated with BBS patients. Future studies are needed to determine how disruption of the BBSome leads to an increase in

angiotensinogen gene expression and whether *in vivo* RAS blockade may protect against the increased arterial stiffness associated with smooth muscle BBSome disruption.

Perspectives

The current study demonstrates for the first time a critical role for the smooth muscle BBSome in the regulation of vascular function and arterial stiffening in a manner independent of blood pressure and body weight. These findings demonstrate a link *between* basic cell biological mechanisms conserved in all organisms (i.e. intracellular transport and cilia function) and complex phenotypes such as cardiovascular dysfunction that result when these mechanisms are perturbed. Additional investigations into the role of the BBSome in mediating cardiovascular dysfunction are warranted. These investigations may help further our understanding of the basic molecular mechanisms that underlie vascular diseases associated with various conditions as well as provide novel therapeutic targets for the clinical treatment of vascular dysfunction and arterial stiffening, two major and independent risk factors for cardiovascular events.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Novelty and Significance

What Is New?

- Smooth muscle-specific BBSome disruption interferes with the aortic vascular relaxation responses.

- Smooth muscle-specific BBSome disruption alters contractile responses in the aorta and mesenteric artery.

- Smooth muscle-specific BBSome disruption increases arterial stiffening and vascular angiotensinogen gene expression.

What Is Relevant?

- The smooth muscle BBSome plays an important role in the regulation of vascular function and the development of arterial stiffness.

- Dysregulation of this protein complex may contribute to the vascular dysfunction associated with various conditions.
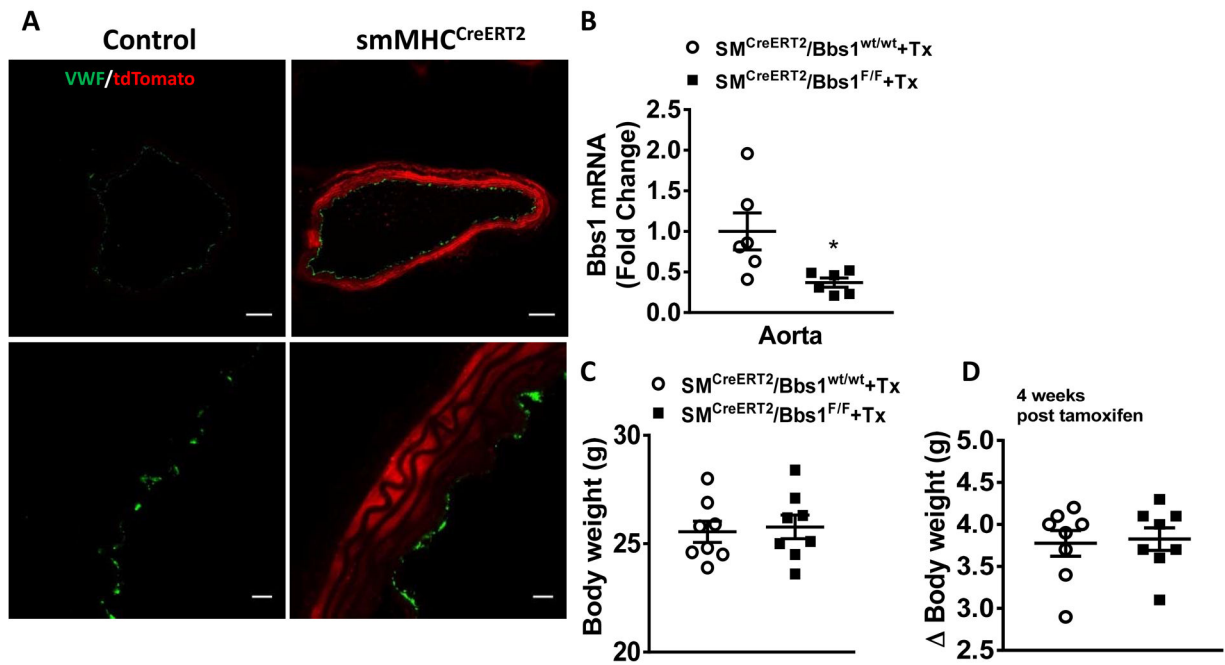
Figure 1 –. Validation of smooth muscle-specific Bbs1 knockout mice.
(**A**) Representative confocal images of aortic rings from tamoxifen (Tx)-treated SM^{CreERT2}/
Bbs1$^{F/F}$ and wildtype mice visualized for td-Tomato (red) and von Willebrand factor (green)
at low (top) and high (bottom) magnification. (**B**) *Bbs1* mRNA expression of aortic rings
from tamoxifen-treated SM^{CreERT2}/*Bbs1*$^{F/F}$ and wildtype mice (n=6/group; *p<0.05 via
unpaired t-test). (**C**) Total body weight and (**D**) weight gain after tamoxifen treatment in
SM^{CreERT2}/*Bbs1*$^{F/F}$ and wildtype mice (n=8/group), bar: 100µm.
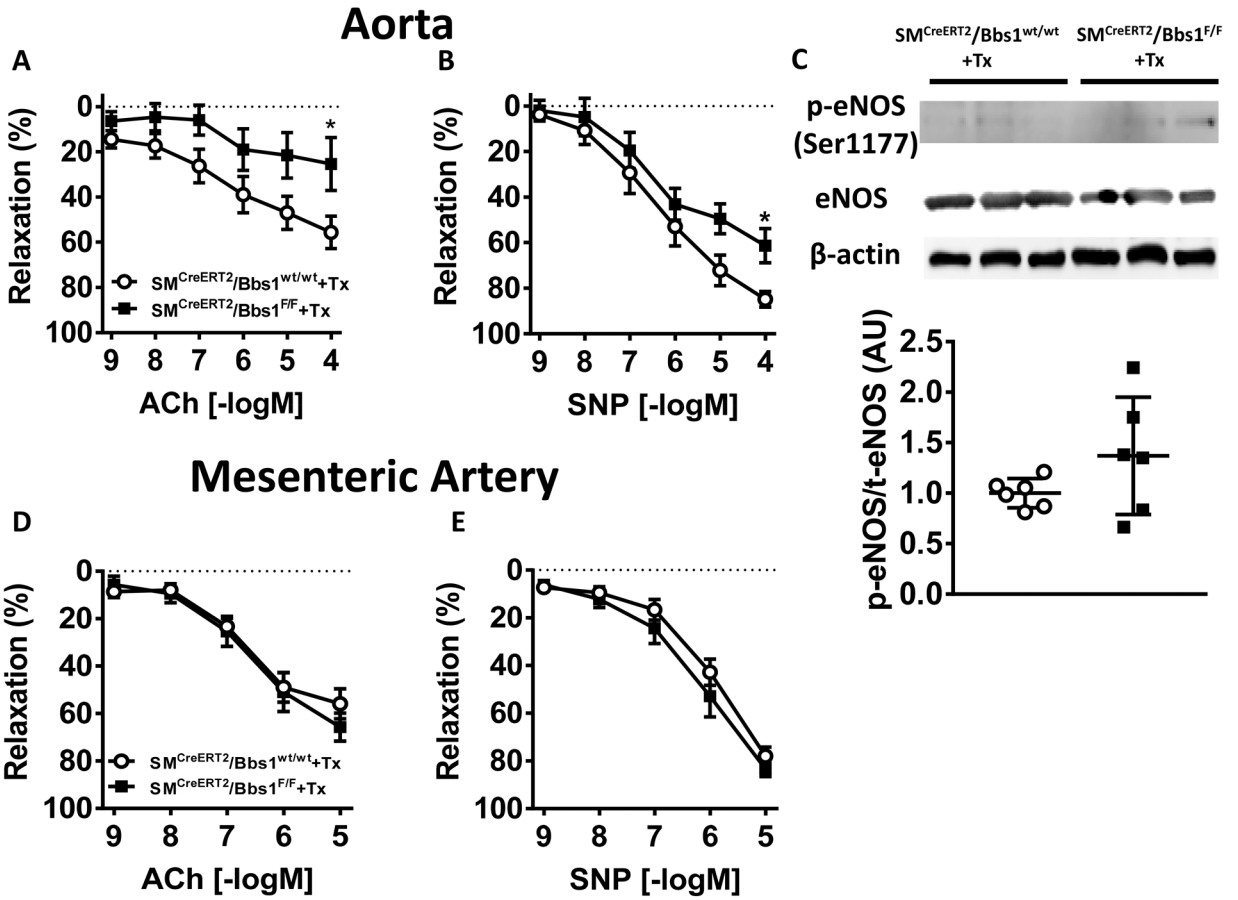
Figure 2 –. Vasorelaxation responses of aortic and mesenteric artery rings from smooth muscle-specific Bbs1 knockout mice.

Cumulative concentration response curves measured in aortic (top) and mesenteric arterial (bottom) rings from tamoxifen-treated SM^CreERT2^/*Bbs1*^F/F^ and wildtype mice. Relaxation responses to acetylcholine (ACh, **A,D**) and SNP (**B,E**) (n=8/group; *$p_{interaction}$<0.05 via 2 way ANOVA with repeated measures). (**C**) Protein expression of aortic rings from tamoxifen-treated SM^CreERT2^/*Bbs1*^F/F^ and wildtype mice for ratio of p-eNOS(Ser1177)/eNOS (n=6/group).
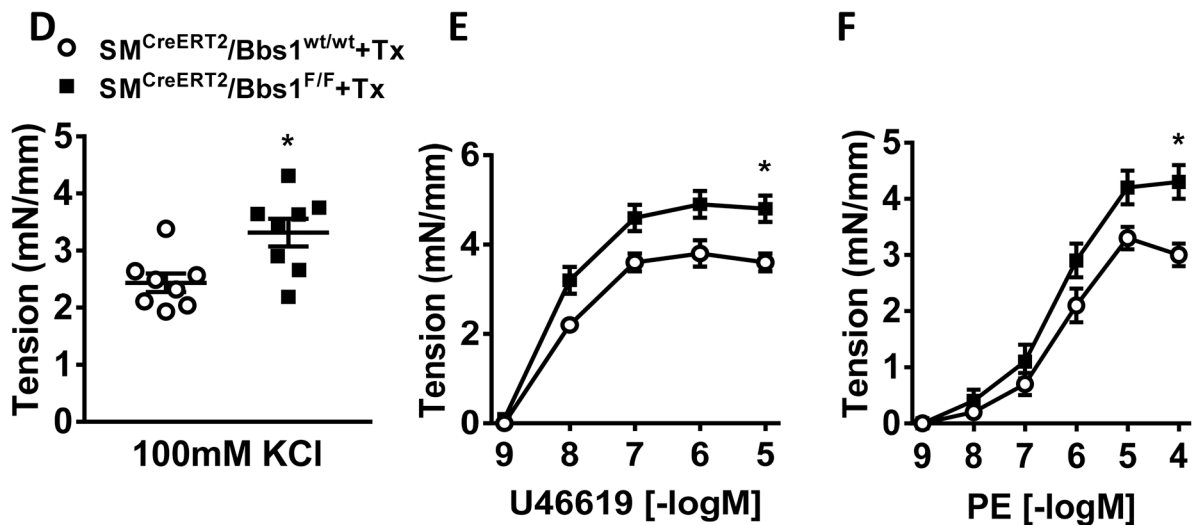
Aorta
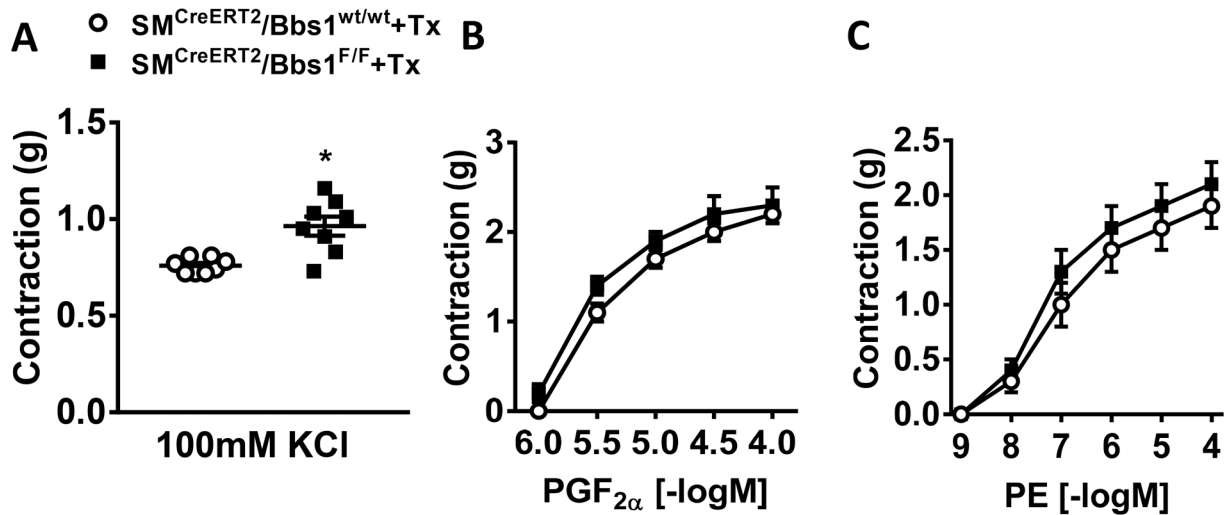
Mesenteric Artery

Figure 3 –. Contractile responses of aortic and mesenteric artery rings from smooth muscle-specific Bbs1 knockout mice.

Cumulative concentration response curves measured in aortic (top) and mesenteric artery (bottom) rings from tamoxifen-treated SM^{CreERT2}/$Bbs1^{F/F}$ and wildtype mice. Contractile responses to KCl (**A,D**), PGF$_{2\alpha}$ (**B**), U-46619 (**E**), phenylephrine (PE; **C,F**). n=8/group; (A,D): *p<0.05 via unpaired t-test; (E,F): *p$_{interaction}$<0.05 via 2 way ANOVA with repeated measures.
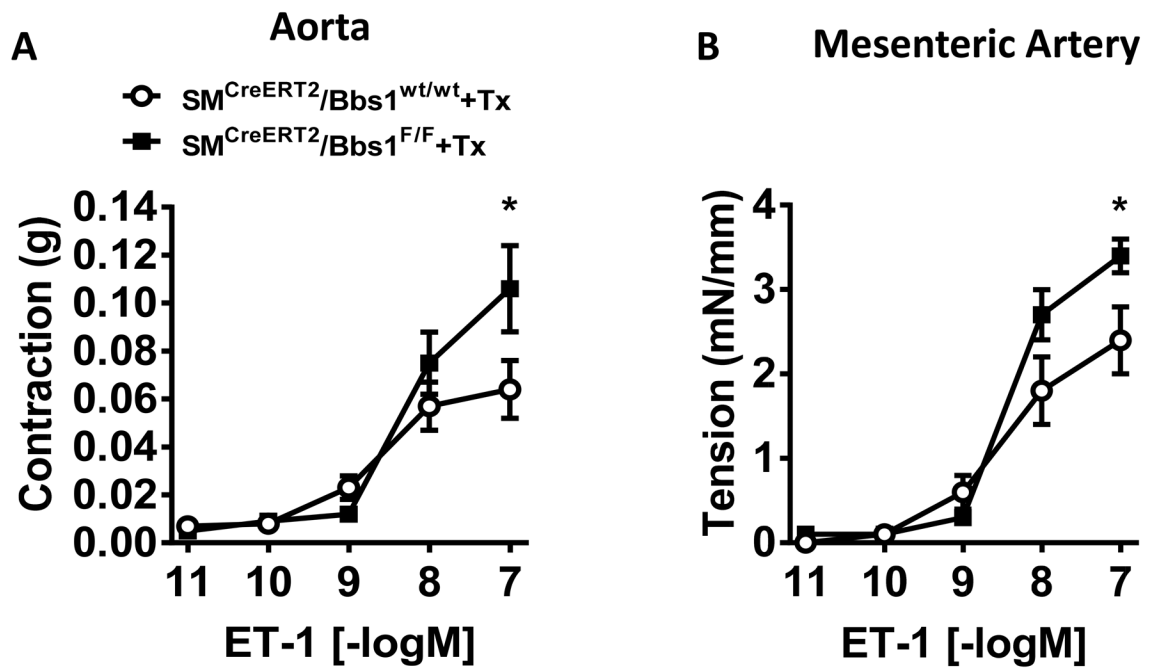
A **Aorta**

B **Mesenteric Artery**

Figure 4 –. Contractile responses to endothelin-1 of aortic and mesenteric artery rings from smooth muscle-specific Bbs1 knockout mice.

Cumulative concentration response curves in aortic (**A**) and mesenteric (**B**) arterial rings to endothelin-1 (ET-1). (n=6–7/group; $*p_{interaction} < 0.05$ via 2-way ANOVA with repeated measures).
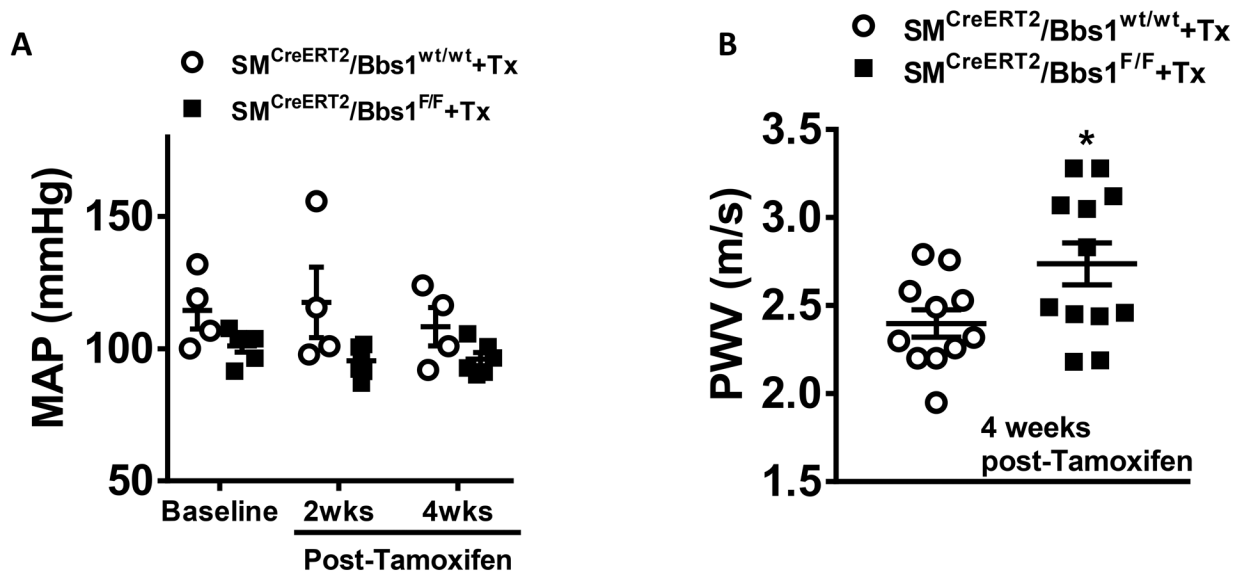
Figure 5 –. Mean arterial pressure and aortic stiffness of smooth muscle specific Bbs1 knockout mice.

Mean arterial pressure (MAP), measured via radiotelemetry, (**A**, n=4–6/group) and pulse wave velocity (PWV), measured via Doppler ultrasound, (**B**, n=11–12/group) in tamoxifen-treated SM^{CreERT2}/$Bbs1^{F/F}$ and wildtype mice at 4 weeks post-Tamoxifen treatment. *p<0.05 via unpaired t-test.
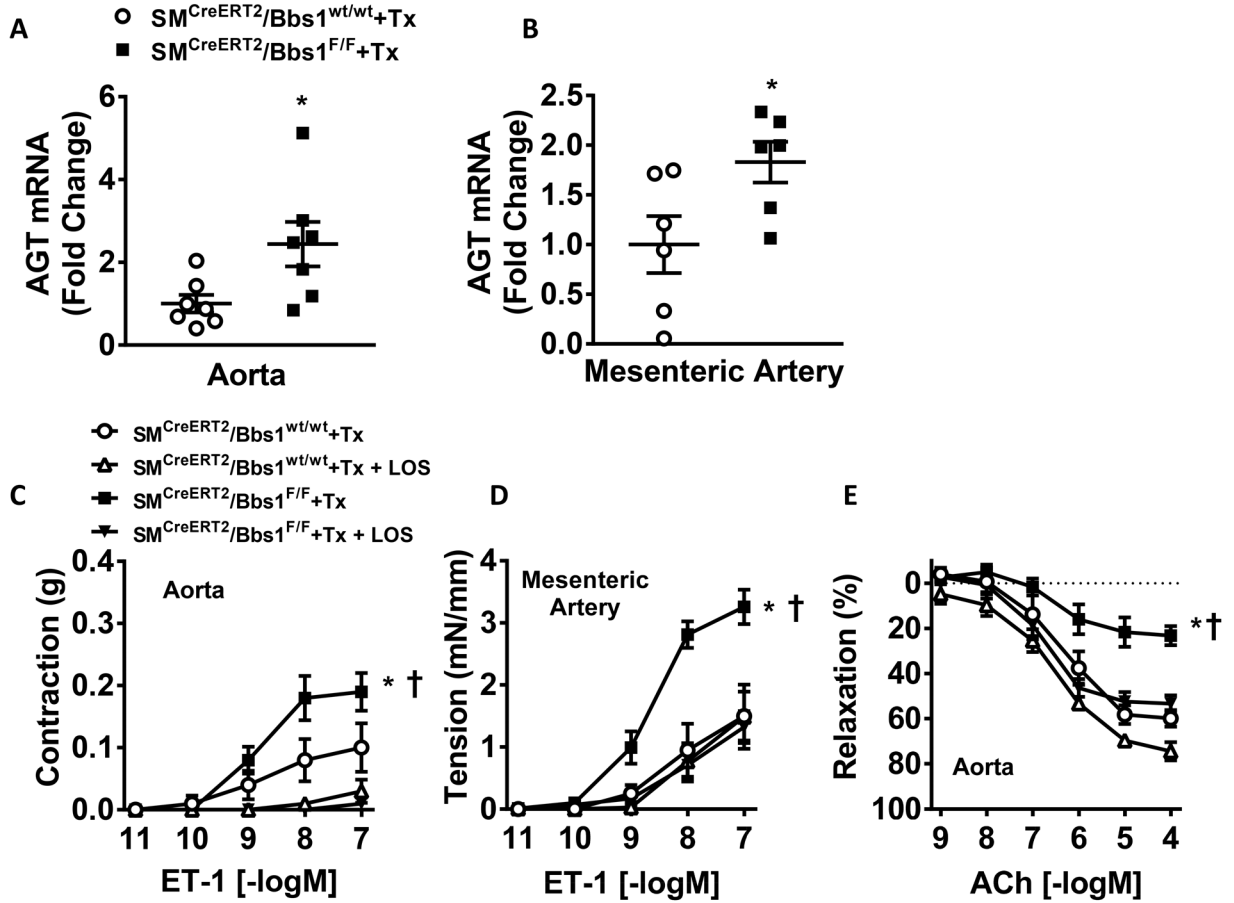
Figure 6 –. Vascular effects of the renin-angiotensin system in aorta of smooth muscle specific Bbs1 knockout mice.

Angiotensinogen expression in aorta (**A**) and mesenteric artery (**B**) of tamoxifen-treated SM^{CreERT2}/*Bbs1*$^{F/F}$ and wildtype mice (n=7/group; *p<0.05 via unpaired t-test). Cumulative concentration response curves in aortic (**C**) and mesenteric arterial (**D**) rings to endothelin-1 (ET-1) with or without losartan pre-incubation (1μM; 30min) (n=6–8/group). Cumulative concentration response curves in aortic rings (**E**) to acetylcholine (ACh) with or without losartan pre-incubation (1μM; 30min) (n=6/group); *†p<0.05 via 2-way ANOVA with repeated measures.