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# Relation between active and passive biomechanics of small mesenteric arteries during remodeling

Bilge Guvenc Tuna [1], Erik NTP Bakker, Ed VanBavel *

*Department of Biomedical Engineering and Physics, Academic Medical Center, PO Box 22700, 1100 DE Amsterdam, The Netherlands*

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ABSTRACT

Small artery remodeling involves matrix reorganization, but may also encompass changed smooth muscle cell biomechanical properties. Here we study the temporal relationship between such contractile plasticity and matrix remodeling in small rat mesenteric arteries subjected to 1 or 3 days of altered flow or acute interventions on matrix structure; cross-linking by transglutaminase and matrix digestion by elastase. Diameter–tension relations were made in the passive state and upon full activation (125 mM $K^+$ and $10^{-5}$ M norepinephrine). In low flow (LF), inward matrix remodeling occurred after 1 day, when the distended diameter at full dilation ($D_{100}$) was reduced from $351 \pm 15$ μm to $299 \pm 14$ μm (SEM, $n=8$, $p < 0.05$). The optimal diameter for force development ($D_{opt}$) was reduced after 3 days, from $291 \pm 10$ μm to $247 \pm 5$ μm (LF, $p < 0.05$). As a result, a mismatch of $D_{opt}/D_{100}$ existed after 1 day of LF, which normalized after 3 days. Dynamics of contraction were studied following quick isometric release by $0.2 \cdot D_{100}$; tension recovery was faster in anatomically smaller vessels following normal flow. This association was partly lost after 1 day of LF, while after 3 days the vessels became not only smaller but also faster, re-establishing this association. High flow vessels demonstrated similar contractile plasticity. Active diameter–tension relations at low distension did not change following transglutaminase or elastase. However, at high distension, any alteration in passive tension coincided with an opposite change in active tension. These data demonstrate an intrinsic interaction between passive and active biomechanics that occurs instantaneously during matrix remodeling at high distensions while contractile plasticity lags matrix remodeling after flow interventions.



## 1. Introduction

Organ perfusion is regulated by the diameter of small resistance arteries. Such control includes acute autoregulation, but also changes in vascular structure and caliber during development. Dysregulation of resistance artery function and structure occurs in a range of cardiovascular disorders, including hypertension (Bakker et al., 2004). In atherosclerosis, vasoregulation of small arteries is impaired due to local endothelial dysfunction and reduction of blood flow and shear stress distal to stenoses (Sorop et al., 2008). A hallmark of such dysregulation is the occurrence of inward small artery remodeling. This limits flow capacity, contributing to compromised organ perfusion and oxygen delivery, heart failure, kidney dysfunction and other target organ damages (Lemarie et al., 2010). Unraveling the processes that determine small artery structure and function in normal life and in pathologies is therefore of key importance for the understanding of cardiovascular disorders. However, despite substantial research, these processes are still only partly understood.

Remodeling of small arteries has frequently been characterized by the change in diameter at full vasodilation, or more extensively by changes in the passive diameter–tension or pressure–diameter relations (Bakker et al., 2006). However, this only addresses the contribution of the passive matrix component. Much less is known on the reorganization of smooth muscle cells (SMC) during remodeling, and on the consequences for active tension-generating capacity. Thus, contractile plasticity of SMC may exist that could be characterized by changes in diameter–tension relations obtained during full contractile activation of the SMC (Tuna et al., 2012). Such plasticity affects the arterial diameter and the range of attainable diameters during normal vasoregulation.

The concept that both passive and active elements rearrange during remodeling is supported by the maintained balance between active and passive biomechanics seen in vessels of widely varying diameter, i.e. during normal development. Thus, peak active tension generally occurs at a diameter ($D_{opt}$) slightly below the passive diameter at 100 mmHg ($D_{100}$), an observation that forms the basis for the 'normalization' procedure in wire myography (Halpern et al., 1978). Some indications for the maintenance of this balance in

* Corresponding author. Tel.: +31 20 5665203/5665200.
*E-mail address:* e.vanbavel@amc.uva.nl (E. VanBavel).
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experimental remodeling come from hypertensive and ligation models (Arner and Uvelius, 1982; Buus et al., 2001). This relation is maintained during development and remodeling by unidentified mechanisms. A possibility is that the $D_{opt}/D_{100}$ balance is an intrinsic property of the wall organization and is therefore continuously maintained during vascular remodeling. Alternatively, SMC plasticity and matrix remodeling are two independent processes with their own dynamics. Indeed, Bakker et al. (2004) showed that overnight activation by endothelin-1 at low distensions induces an inward shift of the active diameter–tension relation in wire-mounted mesenteric small arteries, without changes in matrix biomechanics. Martinez-Lemus et al. (2004) demonstrated early reorganization and relengthening of SMC following vasoconstriction in isolated pressurized vessels. These in vitro data suggest that contractile plasticity may occur early in remodeling, forming a possible step in the observed tight link between maintained contractile activation and matrix remodeling (Bakker et al., 2004; Martinez-Lemus et al., 2004). However, very little information on vascular contractile plasticity and its relation to matrix remodeling is available from in vivo remodeling studies.

Here we study the temporal relationship between contractile plasticity and matrix remodeling in small rat mesenteric arteries subjected to altered flow. We characterized contractile plasticity from the active diameter–tension relation as well as from the dynamics of tension recovery following quick isometric releases. We furthermore tested whether an intrinsic relation between passive and active biomechanics exists by acute interventions on matrix structure, i.e. cross-linking by transglutaminases (van den Akker et al., 2011) and matrix digestion by elastase.

## 2. Materials and methods

Male Wistar rats (Charles River) of 8–12 weeks old, were anaesthetized with isoflurane inhalation (2.5% isoflurane in oxygen during surgery) and placed on a heating pad. Blood flow–modifying surgery was performed in the animals by ligation of second-order mesenteric arteries, a well-established procedure (Bakker et al., 2006; Buus et al., 2001; Hilgers et al., 2010). Three adjacent second-order segments were randomly selected. The two outer segments were ligated with one suture to give low blood flow (LF), while the middle segment received compensating high blood flow, perfusing the distal arcading network (HF) (Fig. 1). After 1 or 3 days of altered blood flow, the rats were sacrificed with an i.p. injection of pentobarbital (100 mg/kg) and decapitated. All experiments were approved by the local committee for animal experiments.

Mesenteric small arteries were dissected and put into cold MOPS buffer of the following composition (in mM) 145.0 NaCl, 4.7 KCl, 1.17 $MgSO_4$, 1.2 $NaH_2PO_4$, $CaCl_2$ 40, 5.0 glucose, and 2.0 pyruvate; pH 7.35. The LF and HF segments were taken upstream of the ligations. 2 mm segments were mounted between an isometric force transducer and a displacement device (Myograph, Danish Myo Technology, Denmark) using two 40 μm wires. The bath solution was changed to physiological saline solution (PSS) of the following composition (in mM): NaCl 119, $NaHCO_3$ 25, KCl 4.7, $KH_2PO_4$ 1.18, $MgSO_4$ 1.17, $CaCl_2$ 2.5, EDTA 0.027, and glucose 5.5, bubbled with 95% air/5% $CO_2$ and kept at 37 °C. The experiments were continuously recorded using Powerlab and the software program Chart (AD Instruments, Hastings, UK).

After acclimatized for 30 min, the inner diameter of the passive vessel at an equivalent pressure of 100 mmHg ($D_{100}$) was determined (Halpern et al., 1978). The vessels were then set to $0.9 \bullet D_{100}$ for 10 min, and stimulated twice by 125 mM $K^+$ and $10^{-5}$ M norepinephrine (NE) to test for viability, followed by a test of endothelium dependent relaxation by $10^{-4}$ M metacholine for 5 min after precontraction with NE ($10^{-5}$ M).

Passive and active diameter–tension relations were determined at 0.4 to $1.1 \bullet D_{100}$, in $0.1 \bullet D_{100}$ steps (Fig. 2). At each distension, passive tension was recorded after at least 3 min of relaxation in PSS and total tension at full activation was determined 5 min after start of stimulation with 125 mM $K^+$ and $10^{-5}$ M NE, when tension had reached steady state. In addition, at $0.7–1.0 \bullet D_{100}$, isometric releases were performed under passive and active conditions by quickly reducing the diameter by 0.2 $D_{100}$ (see Fig. 2) and maintaining this distension for 4 min. In the analysis, we took the value at 4 min as steady state, even though a very minor further increase in tension could be observed, as depicted in Fig. 2. This rapid isometric release was performed by a stepping motor controlled by dedicated software written in Matlab.

These biomechanics were also determined in NF vessels before and after 10 min of digestion by elastase (7.5 μ/ml) or 1.5 h matrix cross-linking by incubation with transglutaminase (TG2, 50 μg/ml) in a reducing environment (TCEP, 1 mM). All chemicals were purchased from Sigma.

Tensions in mN/mm were calculated from the recorded forces and the vessel length. Relaxation to methacholine was presented as fraction of the NE-induced precontraction. Active tension was calculated by subtracting the passive tension from the total tension. The diameter for maximum tension development ($D_{opt}$) was calculated using parabolic curve fitting of the 5 highest points in the active diameter–tension relation. The net active dynamic response after rapid $0.2 \bullet D_{100}$ release was determined by subtracting the passive response from the recorded transient under full stimulation. The dynamics of this response were characterized by double-exponential fitting and are expressed here as the time needed to re-obtain 50% of the tension following the release ($T_{1/2}$).

Statistical analysis was performed using one-way analysis of variance (ANOVA) with Dunnett's post-hoc test by using SPSS 19.0. Data were expressed as mean ± SEM and differences were considered statistically significant at $p < 0.05$.

## 3. Results

One day after ligation, contractile responses to KPSS+NE under low flow (LF), normal flow (NF) and high flow (HF) were similar (LF; 17.0 ± 1.5 mN, NF; 18.1 ± 1.4 mN, HF; 17.2 ± 1.4 mN, $p > 0.05$, $n=8$). After 3 days, LF vessels had slightly and not significantly smaller maximum contractile responses compared with NF, while HF arteries had slightly larger responses (LF; 17.5 ± 0.8 mN, NF; 19 ± 0.6 mN, HF; 21.4 ± 0.9 mN, $p > 0.05$, $n=8$). Endothelial function, as assessed by methacholine after precontraction with NE, was not different between NF, LF and HF (1 day; 52.8 ± 10.4%, 66.3 ± 11.1%, 84.3 ± 7.8%, $p > 0.05$, 3 days; 88.8 ± 6.6%, 83.7 ± 6.3%, 81.3 ± 7.4%, $p > 0.05$, $n=8$).

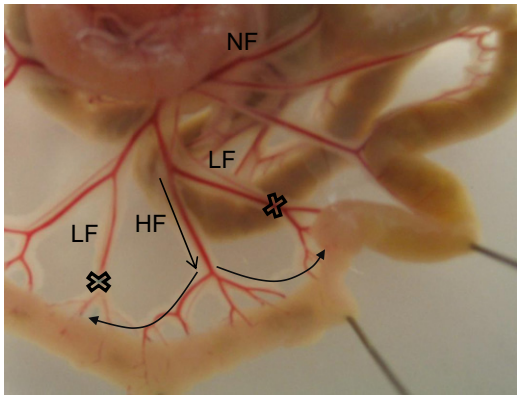


**Fig. 1.** The mesenteric bed with a schematic illustration of the ligations (crosses) that were made to create low flow (LF) and high flow (HF) vessels, the location of normal flow (NF) arteries, and the flow from the HF vessel, via the small arcading arteries (arrows).

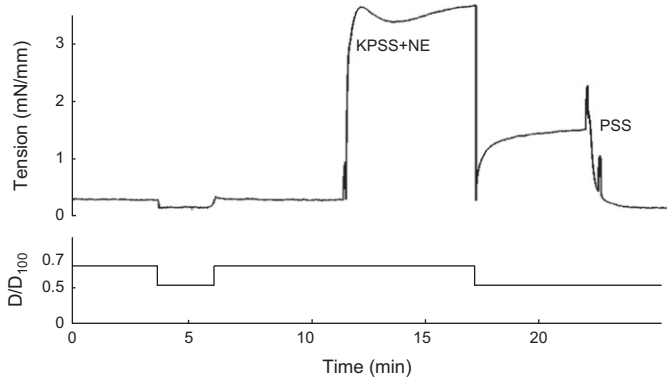


**Fig. 2.** Example of the responses of a wire-mounted rat mesenteric artery after 1 day of NF. The vessel was pre-stretched to $0.7 \bullet D_{100}$, subjected to a release to $0.5 \bullet D_{100}$, followed by restretch, stimulation by 125 mM $K^+$ and 10 μM NE, and a release under these active conditions.