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Increased stiffness and cell-matrix interactions of abdominal aorta in two experimental non-hypertensive models: long-term chemical sympathectomized and sinoaortic-denervated rats

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Short title: Aorta structure and stiffness in rats

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equal contribution

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ABSTRACT

RATIONALE: Sinoaortic denervated (SAD) and chemically sympathectomized (SNX) rats are characterized by a decrease in arterial distensibility without hypertension and would thus be relevant for analyzing arterial wall stiffening independently of blood pressure level. The fibronectin network, which plays a pivotal role in cell matrix interactions, is a major determinant of arterial stiffness. We hypothesized that in SAD and SNX rats, arterial stiffness is increased, due to alterations of cell-matrix anchoring leading to spatial reorganization of the extracellular matrix.

METHODS: The intrinsic elastic properties of the arterial wall were evaluated in vivo by the relationship between incremental elastic modulus determined by echotracking and circumferential wall stress. The changes of cell-extracellular matrix links in the abdominal aorta were evaluated by studying fibronectin, vascular integrins receptors and ultrastructural features of the aorta by immunochemistry.

RESULTS: In both experimental conditions wall stiffness increased, associated with different modifications of cell-extracellular matrix adhesion. In SAD rats, increased media-cross sectional area was coupled with an increase of muscle cell attachments to its extracellular matrix via fibronectin and its α5-β1 integrin. In SNX rats, reduced media-cross sectional area was associated with up-regulation of αv-β3 integrin and more extensive connections between dense bands and elastic fibers despite the disruption of the elastic lamellae.

CONCLUSION: In aorta of SNX and SAD rats, a similar arterial stiffness is associated to different structural alterations. An increase in αvβ3 or α5β1 integrins together with the already reported increase in the proportion of less distensible (collagen) to more distensible (elastin) components in both models, contribute to
remodeling and stiffening of the abdominal aorta.
CONDENSED ABSTRACT

Sinoaortic denervated (SAD) and chemically sympathectomized (SNX) rats are models of decreased arterial distensibility without hypertension allowing analyzing arterial stiffening and structure independently of blood pressure level. Increase in arterial wall stiffness in both models was associated with structural alterations. In SAD, increased media cross sectional area was coupled with increased muscle cell attachments via fibronectin and α5-β1 integrin. In SNX, reduced media cross sectional area was associated with up-regulation of αv-β3 integrin and alteration of elastin fibers. In these rats, similar arterial stiffness in absence of hypertension is associated to differential structural alterations.

**Key words:** Sino-aortic denervation; Sympathectomy; fibronectin; Arterial Stiffness, Integrins
INTRODUCTION

Increased stiffness of large arteries is a significant and independent predictor of cardiovascular (CV) diseases [1]. Arterial stiffness is evaluated by the elastic properties of the artery as a whole measured by arterial distensibility and by the elastic properties of the arterial wall material measured by the incremental elastic modulus (Einc) [2]. Arterial distensibility and Einc are then 2 complementary parameters used to describe arterial stiffness. Until now, it remains difficult to separate the causal effects of blood pressure elevation from that of the mechanical and functional properties of the arterial wall that lead to alterations of distensibility and stiffness. We have previously shown that the spontaneously hypertensive rat (SHR) is characterized by a decreased distensibility at its operational pressure compared to its normentensive control [3]. Nevertheless evaluation of the arterial wall stiffness, assessed by the elastic modulus measurement, shows that for a given level of stress, SHR and Wistar rats have similar mechanical properties. These results indicate that the decrease of distensibility observed in SHR is related to hypertension, rather than to increased stiffness of the arterial wall. Therefore, other experimental models must be used to analyze the intrinsic stiffness of the aortic tissue. Sinoaortic denervated (SAD) rats and chemically sympathectomized (SNX) rats should help to investigate this issue. Both models are characterized by a decrease in arterial distensibility without hypertension compared to their respective control [4, 5], suggesting that they would be relevant for analyzing arterial wall stiffening independently of blood pressure level.

The fibronectin (Fn) network, which plays a pivotal role in cell matrix interactions, is a major determinant of arterial stiffness [3, 6]. Fibronectin controls deposition and organization of extra cellular matrix and modulates both cell proliferation and vascular
smooth muscle cell (SMC) phenotype. Thus, by increasing cell-matrix anchoring through a α5-β1-integrin, aortic fibronectin accumulation may contribute to protect the arterial wall components from the increased mechanical loads associated with hypertension in young and old SHRs [3]. The accumulation of other integrins, such as αv-β3 integrin, has also been observed in the mesenteric artery of SHR [7]. These results suggest that cell-matrix interactions, which play a major role in SMC function, are also involved in the mechanical properties of the vascular wall. To our knowledge, cell-matrix interactions have never been described in non-hypertensive models related to arterial stiffness.

We hypothesized that in SAD and SNX rats, arterial stiffness is increased, and this may be due to alterations of cell-matrix anchoring leading to spatial reorganization of the extracellular matrix. We aim to determine in SAD and SNX rats (i) the intrinsic elastic properties of the arterial wall by evaluating in vivo the relationship between Einc and circumferential wall stress, and (ii) the changes of cell-extracellular matrix links in the abdominal aorta, by studying fibronectin, vascular integrins receptors and ultrastructural features of the aorta.

MATERIALS AND METHODS

Animals

Male Wistar rats (Iffa-Credo, Fresnes, France) were used. All procedures were in accordance with institutional guidelines for animal experimentation and conformed to the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health.
Sino-aortic denervation and SNX were performed as previously described [4, 5]. In brief, SAD was performed at 10 weeks of age on anesthetized rats. Sham-operated rats were used as control of SAD rats. All the rats of these groups were examined and killed at 16 weeks of age. SNX rats were sympathectomized by subcutaneous injections of 50 mg/kg guanethidine sulfate for 12 weeks, 5 times a week, from day 5 after birth. The control (CO) rats received saline injections according to the same schedule, and both sets of rats were investigated during their 13th week of age. A total of 27 rats were used for the in vivo experiments and 38 rats for immunohistochemistry and electron microscopy.

**Hemodynamic investigations**

Mechanical properties of the abdominal aorta were assessed by circumferential wall stress ($\sigma$) and $E_{inc}$. At the end of the treatment, under pentobarbital anesthesia (60 mg/kg ip), a catheter was introduced in the lower abdominal aorta via the femoral artery for blood pressure recording. A midline laparotomy was then performed and the probe of the ultrasonic device (NIUS-01, Asulab SA) positioned 1 cm above the aortic bifurcation for recording of internal arterial diameter. Blood pressure and internal arterial diameter were then simultaneously recorded. The relationship between pressure and lumen cross-sectional area was calculated by means of an arctangent function. $\sigma$ and $E_{inc}$ were calculated with the above-mentioned parameters and medial cross-sectional area of the aorta determined by histomorphometry as previously described [3, 6, 8].

**Antibodies**
The antibodies used were monoclonal mouse antibodies (mAbs) reactive with an alternatively spliced form of fibronectin, EIIIA-Fibronectin (clone IST-9, Valbiotech, France) and all FN isoforms (Total-Fn, Valbiotech, France) [3], a mouse anti-vimentin monoclonal antibody (1/400, clone V9, Dako, France), a mouse anti-actin monoclonal antibody (1/500, clone 1A4, Dako, France), a rabbit anti-integrin αv polyclonal antibody (1/250, chemicon) a rabbit anti-integrin α5 subunit polyclonal antibody (Valbiotech, France) [3].

**Immunohistochemical investigation**

Immunohistochemical staining was performed on fresh unfixed freeze-dried suprarenal abdominal aorta [3]. We used the indirect immunoperoxidase technique as previously described for the determination of fibronectin, the EIIIA-Fibronectin isoform and the α5 integrin [3, 9]. The determination of actin, vimentin, αv integrin were performed on a Dako automate as described elsewhere [10]. No specific staining was observed when primary antibody was omitted from the protocol (negative control). The distribution and quantification of staining were determined by computer-directed color analysis performed with the Quant’Image software (Quancoul, Talence, France) [3].

**Electron microscopy**

The thoracic aortas (2 SAD, 2 SNX and 5 controls) were fixed in situ by transcardiac perfusion of fixative (4% glutaraldehyde and 1% formaldehyde in 0.1M Na-cacodylate). Thin (0.5-3.0 µm) and ultrathin (100 nm) sections were cut on a plane transverse to the thickness of the media and parallel to the length of the vessel, an approach that produced approximately transverse sections of the muscle cells. The sections were
stained with uranyl acetate and lead citrate and viewed in a Philips 400 microscope. Photographic montages were made, covering the thickness of the media over a length of 150-400 µm, at a magnification of 8000x. On these montages the size of nucleated muscle cell profiles was measured as well as the percentage of the cell membrane displaying dense bands and the percentage of cell membrane in contact with lamellae of elastin [8]. Ultrastructure characterization and quantification were evaluated by counting tissue points of randomly selected photographic fields and a minimum of 6 nucleated cells per rat were quantified as previously reported [8]. Quantification of elastin and collagen have been presented elsewhere [4, 5].

**Statistical Analysis**

All values were averaged and expressed as mean ± SEM. Unpaired Student’s t tests were performed to compare SNX and SAD rats with their respective controls for arterial and immunohistochemical parameters [3]. For statistical comparison of Einc-σ curves between groups, Einc was log transformed to generate linear relationships. Calculating the r2 of the linear regression obtained with the new parameters for each individual checked the quality of the transformation. After this transformation, we calculated the mean slopes of the curves. If the slopes were not significantly different, we compared the curves by calculating the mean wall stress at 800 kPa of Einc (σ800), a value common to all groups [11]. Differences were considered significant at values of P<0.05.

**RESULTS**

*Hemodynamic and aortic mechanics*
Body weight of SNX rats was significantly lower than that of control rats (303 ± 10 g vs. 353 ± 3 g, p<0.05). Likewise, SAD rats were significantly lighter than sham-operated rats (428 ± 13 g vs. 464 ± 7 g, p<0.05). Compared to their respective controls, heart rate (data not shown) and mean arterial pressure (MAP) were significantly reduced in SNX rats but remained unchanged in SAD rats. Media cross sectional area was significantly reduced in SNX rats compared to their controls; but was significantly increased in SAD rats compared to sham-operated rats. Therefore, SAD rats had significantly higher MCSA than SNX rats. Einc at MAP remained unchanged in all groups of rats. The circumferential wall stress was similar in SNX and SAD rats both at MAP and at 800 kPa of Einc but was significantly reduced when compared to their respective controls (Table 1). The Einc-wall stress curves of SNX and SAD rats were similarly shifted leftwards compared with their controls, indicating increased stiffness of the wall in both models (Figure 1).

**Immunohistochemistry**

Total-fibronectin and α5 integrin subunit staining were diffuse in the media of all control rats (Figures 2 and 3). Staining for both components was markedly increased in SAD rats compared to Sham-operated rats (Figure 2) whereas no accumulation of fibronectin and its α5 integrin were found in SNX rats compared to their controls (Figure 3).

In Sham-operated and CO rats, immunoreactivity for cellular fibronectin (EIIIA-fibronectin), was observed in the inner part of the media. In SAD rats the EIIIA-fibronectin staining was equally intense but it involved the entire thickness of the media
A slight increased of EIII-A fibronectin was found in SNX rats compared to CO (Figure 3).
The endothelium highly expressed αv staining and the media showed relatively low but extensive expression in all control rats. In contrast with α5 integrin, αv and vimentin staining were increased in SNX rats (Figure 3) but unchanged in SAD rats (Figure 2) compared to their respective controls. Immunostaining for actin was equally intense in all conditions.

Electron Microscopy

The intima of the rats’ aortic wall is composed of a thin endothelium, sub-endothelium connective tissue and an inner elastic lamina. The endothelium of the thoracic aorta appeared similar in all groups.
The media, composed of elastic lamellae, with interposed layers of muscle cell and interconnected by elastic fibers and bundles of collagen fibrils, showed some alterations in the experimental conditions; however, the amount of elastic material in the wall appeared unchanged. In SNX rats some disruption of the elastic lamellae was observed, including the breaking up of some lamellae into large elastic bundles (Figure 4A). The muscle cells profiles, observed in transverse section, had an irregular contour, with large processes and invaginations, in all preparations. Numerous dense bands associated with actin bundles on the cytoplasmic side and with collagen and elastic fibers extracellularly, occupied the muscle cell membrane.

Despite the restricted number of animals used per groups, results observed were quite reproducible as already validated [8] and allow pooling data from sham-operated rats and control of SNX rats. In control rats, dense bands occupied 42 ± 2 % of the cell
perimeter and half of it (20% ± 2 %) was connected to elastic fibers. The number of dense bands appeared obviously increased in SAD rats (57% ± 3 %; p<0.05) compared with controls but not in SNX rats (45% ± 2 %). In the latter there were more extensive connections between dense bands and elastic fibers than in controls (30 ± 2 %; p<0.05). The percentage of dense bands connected to elastic lamellae remained unchanged in SAD rats (Figure 4B).

**DISCUSSION**

In the present study, structural changes of the abdominal aorta were evaluated in SNX and SAD rats, two models of decreased arterial distensibility without hypertension. We observed an increase in wall stiffness in both experimental conditions, but different structural changes in the vessel wall. In SAD rats, aortic hypertrophy was coupled with an increase of muscle cell attachments to its extracellular matrix via fibronectin and its α5-β1 integrin. In SNX rats, aortic hypotrophy was associated with αv-β3 integrin up-regulation and alteration of elastin fibers.

In contrast to acute treatment, chronic treatment with guanethine significantly reduced blood pressure as already reported [5, 12]. This effect may be due to the succession of many hypotensive episodes, previously reported in this model [13]. A weight loss was also observed as previously reported in both models [12].

We have previously shown a similar reduction of carotid distensibility in SAD and SNX rats [4, 5]. While arterial distensibility is an indicator of the elastic properties of the artery as a hollow structure, the Einc expresses the elastic properties of the wall material that is independent of wall intima-media thickness [6]. Enhanced aortic stiffness is a significant and independent risk factor for all-cause and cardiovascular mortality [1],
primarily coronary heart disease [14] and stroke [15] in human. Thus, elaboration of Einc/stress curves addresses arterial wall stiffness, independently of the wall thickness and of the pressure level. To our knowledge, stiffness of the arterial wall material had never been evaluated and compared in SNX and SAD rats. Therefore, the first new finding of the present experiments is that SNX and SAD rats are characterized by a similar increase in arterial wall stiffness (leftwards shift of the Einc-stress curves).

We have previously shown that in SHRs, the Einc of the aortic wall material, determined for a given level of circumferential wall stress, was not significantly different from that of Wistar rats. This indicates that arterial wall materials in SHR and its control strain have similar mechanical behavior [3]. In contrast, the increased stiffness of the arterial observed in the present study suggest that SNX and SAD rats seem pretty relevant models for analyzing arterial remodeling associated with stiffness.

The second new finding of the present study derived from the characterization of extracellular matrix changes in both models. Extracellular matrix proteins determine the passive biomechanical properties, collagen providing tensile strength and elastin enabling vascular elasticity [16]. Indeed we and others have shown a strong relationship between decreased elastin/collagen ratio and arterial stiffness in both models indicating an alteration in the organization of the ECM [4, 5, 17]. However, because SNX is characterized by reduced MCSA (present results) with a predominant reduction in elastin [5], and SAD by an increased MCSA (present results) and a predominant increase in collagen [4], a different structure-function relationship is present in the two experimental conditions.

The dense bands of muscle cells provide a link between contractile apparatus and extracellular matrix, mediated by integrin receptors on the cell membrane [8, 18, 19].
rat aorta, the major integrins ligands are fibronectin, a glycoprotein that plays an important role in the organization and assembly of the extracellular matrix, collagen and laminin. Accumulation of collagen in the aorta of SAD rats is associated with accumulation of total-fibronectin and its α5β1-integrin receptor, indicating an increased mechanical linking/coupling between muscle cells and extracellular matrix [19-21]. Alteration of cell-matrix attachments might thus contribute to increase arterial stiffness, as already reported in SHRs [3]. This result is strengthened by the obvious ultrastructural changes of the aorta shown in the present study where the number of dense bands per muscle cell profile is enhanced. In SAD rats, extracellular matrix composition is also characterized by an accumulation of EIIIA-fibronectin, up regulated during hypertension and aging [3, 21, 22], and closely associated with arterial stiffness [3, 6, 8, 23]. Despite the disruption of the elastic lamellae, we also observed an increase of cell-elastin connections and accumulation of αvβ3 integrin and vimentin in SNX rats. It is now well established that many αvβ3 integrin-rich focal are associated with vimentin intermediate filament cytoskeletons in parallel [24, 25]. Therefore, the accumulation of vimentin observed in SNX rats is in good agreement with αvβ3 integrin up-regulation. We and others have already observed enhancement of ultrastructural connections of smooth muscle with elastin in rat vessels, as reported in the present study [8, 26]. We suggest that αvβ3 integrin accumulation is mirrored by increase in the spatial density of dense bands observed. It should contribute to add strength to the structure of the vascular wall through focal attachments of vascular SMC with extracellular matrix. Aside from acting as a physical joint, αvβ3 integrins may also promote vascular remodeling. The isolated increase of EIIIA-fibronectin associated with αvβ3 and
vimentin accumulation, already reported in hypertensive rats, is associated with
eutrophic inward remodeling of small arteries [27]. It is well established that arterial
total-fibronectin content increases with increased arterial pressure. Nevertheless, the
small increase of EIII-A fibronectin observed is independent of the blood pressure level,
as guanethine significantly reduced blood pressure in SNX rats compared to control.
Our data support the concept that sympathectomy favors the expression of the immature
phenotype of smooth muscle [28-30].
Beside hypertension and vascular disease such as atherosclerosis, increase blood
pressure variability might be a possible mechanism of increase arterial stiffness as
recently reported in human [31-33] and rats [15]. Indeed, we and other have shown that
both models are characterized by an increase in blood pressure variability [4, 5, 12, 34].
Blood pressure variability leads to the mechanical process of fatigue, which might be
buffered by modification in cell to matrix interactions. This contributes to the
maintenance of aortic structure through morphological changes that take place in the
vessel wall. The activation of the renin-angiotensin system and the central
noradrenergic neurons described after long-term sino-aortic denervation [35], lead to
vascular hypertrophy through fibronectin-α5 integrin complex. In the opposite, SNX
rats are characterized by aortic catecholamine depletion after chemical sympathectomy
[36]. Arterial wall hypotrophy is associated with serious alterations of the vessel
integrity and elastin alteration as widely observed with aging [37], despite the up-
regulation of αvβ3 integrin [38].
The presented data show the interplay between structure and mechanics of abdominal
aorta in SNX and SAD rats. In the 2 models, increase in αvβ3 or α5β1 integrins
together with the already reported increase in the proportion of less distensible
(collagen) to more distensible (elastin) components plays a key role in remodeling and stiffening of the abdominal aorta.
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References


[7] Intengan HD, Thibault G, Li JS, Schiffrin EL. Resistance artery mechanics, structure, and extracellular components in spontaneously hypertensive rats: effects of
angiotensin receptor antagonism and converting enzyme inhibition. Circulation. 1999 Nov 30;100(22):2267-75.


Martinez-Lemus LA, Hill MA, Meininger GA. The plastic nature of the vascular wall: a continuum of remodeling events contributing to control of arteriolar diameter and structure. Physiology (Bethesda, Md. 2009 Feb;24:45-57.


Table 1: Arterial properties of abdominal aorta in sinoaortic denervated (SAD) and in chemical sympathectomized (SNX) rats.

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<th></th>
<th>Sham</th>
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<th>SNX</th>
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<tr>
<td>Number</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>5</td>
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<tr>
<td>MAP, mmHg</td>
<td>121 ± 2</td>
<td>116 ± 8</td>
<td>113 ± 3</td>
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<td>Media thickness, µm</td>
<td>81 ± 1</td>
<td>79 ± 1</td>
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<td>MCSA, mm²</td>
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<td>0.22 ± 0.01 $</td>
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<td>E_{inc} at MAP, kPa</td>
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<td>760 ± 180</td>
<td>660 ± 40</td>
<td>570 ± 90</td>
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<td>σ at MAP, kPa</td>
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<td>152 ± 20*</td>
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<td>120 ± 12*</td>
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<td>σ at E_{inc=800}, kPa</td>
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<td>172 ± 19*</td>
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<tr>
<td>E_{inc} /σ</td>
<td>204 ± 9</td>
<td>176 ± 32</td>
<td>265±32</td>
<td>228 ± 37</td>
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</table>

Values are mean ± SEM. CO, control of SNX rats; MAP, mean arterial pressure; MCSA, medial cross sectional area; E_{inc}, incremental elastic modulus; σ, circumferential wall stress. *, P<0.05 compared to Sham-operated or CO rats; $, P<0.05 between Sham-operated rats and CO; †, P<0.05 between SAD and SNX.
Figure Legends

Figure 1
Mean aortic Einc-wall stress curves in chronic sinoaortic denervated (SAD) and chronic sympathectomized (SNX) rats and their respective control (Sham and CO). Each point is the mean ± SEM.

Figure 2
Aortic immunostaining of total fibronectin, EIIIA-fibronectin, α5 and α v integrins, smooth muscle alpha actin and vimentin of sinoaortic denervated (SAD) rats and their controls (Sham). Bottom panel presents the quantification of the immunostanining expressed in percent changes over Sham-operated rats. Each bar is the mean±SEM of 5-9 rats. * P<0.05 vs. SHAM.

Figure 3
Aortic immunostaining of total fibronectin, EIIIA-fibronectin, α5 and α v integrins, smooth muscle alpha actin and vimentin of sympathectomized (SNX) rats and their controls. Bottom panel presents the quantification of the immunostanining expressed in percent changes from controls. Each bar is the mean±SEM of 5-9 rats. * P<0.05 vs. controls.

Figure 4
Electronic microscopy of elastic lamellae (A) and smooth muscle cell (B) of the aorta of sinoaortic denervated (SAD), control (data pooled from sham-operated and control of SNX rats) and sympathectomized (SNX) rats.
A- The elastic lamellae are bridged by elastic fibers and are separated by muscle cells and bundles of collagen fibrils. The interlamellar space, defined as the space between
consecutive lamellae, is increased on average in SAD rats. The elastic lamellae are thinner and altered in SNX rats, in which some lamellae appear broken into large elastic bundles.

B- Dense bands are a prominent feature in the media of the rat aorta. In SAD rats, the percentage of cell surface occupied by dense bands is increased compared with sham-operated rats. Dense bands connected to elastic lamellae remain unchanged. In SNX rats, cell surface occupied by dense bands is well conserved. Nevertheless, the percentage of cell surface connected to the elastic lamellae is twice as high in SNX rats compared with their controls.

C- Characterization of Dense bands

Representative images of dense bands associated with collagen (full arrows) and elastic fibers (dashed arrows)
Bouissou et al., Figure 1

![Graph showing the relationship between Einc (kPa) and σ (kPa) for different groups (SAD, SHAM, SNX, CO).](image-url)
Bouissou et al., Figure 3

Percent changes from Co-SNX

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<th>Total fibronectin</th>
<th>EIIIA-fibronectin</th>
<th>α5-integrin</th>
<th>αv-integrin</th>
<th>Actin</th>
<th>Vimentin</th>
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