The role of type I collagen in aortic wall strength with a homotrimeric $[\alpha1(I)]_3$ collagen mouse model

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Purpose: **Elastin and collagen (types I and III) are the primary load-bearing elements in aortic tissue. Deficiencies and derangements in elastin and type III collagen have been associated with the development of aneurysmal disease. However, the role of type I collagen is less well defined. The purpose of this study was to define the role of type I collagen in maintaining biomechanical integrity in the thoracic aorta, with a mouse model that produces homotrimeric type I collagen $[\alpha1(I)]_3$, rather than the normally present heterotrimeric $[\alpha1(I)]_2$ $\alpha2(I)$ type I collagen isotype.**
Methods: **Ascending and descending thoracic aortas from homozygous (*oim/oim*), heterozygous (*oim/+*), and wildtype (+/+) mice were harvested. Circumferential and longitudinal load-extension curves were used as a means of determining maximum breaking strength (F_{max}) and incremental elastic modulus (IEM). Histologic analyses and hydroxyproline assays were performed as a means of determining collagen organization and content.**
Results: **Circumferentially, the ascending and descending aortas of *oim/oim* mice demonstrated significantly reduced F_{max}, with an F_{max} of only 60% and 23%, respectively, of wildtype mice aortas. *Oim/oim* descending aortas demonstrated significantly greater compliance (decreased IEM), and the ascending aortas also exhibited a trend toward increased compliance. Reduced breaking strength was also demonstrated with longitudinal extension of the descending aorta.**
Conclusion: **The presence of homotrimeric type I collagen isotype (absence of $\alpha2(I)$ collagen) significantly weakens the aorta. This study demonstrates the integral role of type I collagen in the biomechanical and functional properties of the aorta and may help to elucidate the role of collagen in the development of aneurysmal aortic disease or dissection. (J Vasc Surg 2001;33:1263-70.)**

Elastin and collagen are the primary extracellular matrix components of the aortic wall, providing the integrity to withstand the outward forces exerted by arterial pressure.[1,2] These elements make up approximately 50% of the dry weight of normal arteries.[1] The dominant component in the thoracic aorta is elastin (approximately 60%), whereas the composition is reversed in the abdominal aorta (70% collagen).[1] Arterial collagen is composed predominantly of type I and type III.[3]

The normal type I collagen isotype is a heterotrimeric molecule, composed of two $\alpha1(I)$ chains and one similar, but genetically distinct, $\alpha2(I)$ chain.[4,5] The homotrimeric type I collagen isotype, composed of three $\alpha1(I)$ chains was originally discovered in certain tumors and cultured cancer cell lines.[6-8] Since then, homotrimeric type I collagen has been shown to naturally occur at low levels in normal adult skin, during embryonic development, and during wound healing.[9-11] Homotrimeric type I collagen has also been associated with certain forms of Ehlers-Danlos syndrome (EDS)[12-14] and osteogenesis imperfecta (OI).[15,16]

Altered elastin content and structure have been implicated in the formation of abdominal aortic aneurysms.[2,17] Because the tensile strength of collagen (1.0×10^9 N/m^2) is approximately 1000 times greater than that of elastin (4.6×10^5 N/m^2), defects in collagen should alter vascular integrity and contribute to aneurysmal development.[18] Defects in type III collagen, responsible for EDS type IV, have a strong association with aortic aneurysmal degeneration.[19-21] However, the role of type I collagen in aortic wall integrity and aneurysmal development remains to be elucidated. This study investigated the impact of the absence of the $\alpha2(I)$ collagen chain and/or the presence of homotrimeric type I collagen $[\alpha1(I)]_3$ on the biomechanical properties of the thoracic aorta to begin to elucidate the causative role and/or contributory role of type I collagen defects and/or polymorphisms in the development of vascular disease.

MATERIAL AND METHODS

Animals. Mice who were homozygous for the homotrimeric type I collagen (*oim/oim*), heterozygous mice (*oim/+*), and wildtype mice (+/+) were purchased from Jackson Laboratory (Bar Harbor, Me). Additional

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Competition of interest: nil.
A portion of this study was presented at the Seventh International Conference on Osteogenesis Imperfecta, Montreal, Canada, Aug 29–Sep 1, 1999.
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doi:10.1067/mva.2001.113579

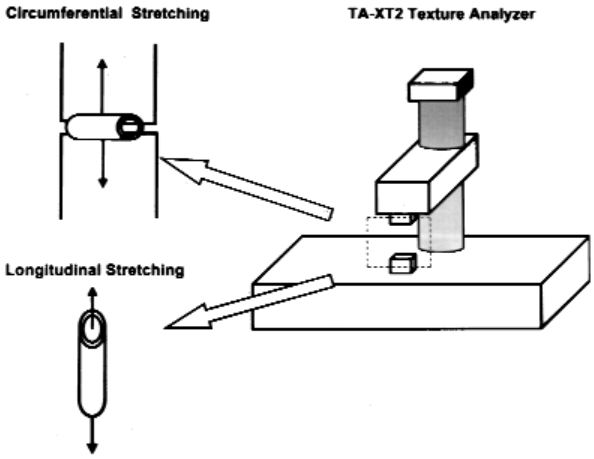

Fig 1. Schematic representation of TA-XT2 texture analyzer system used for load-extension studies of the aortic samples. The TA-XT2 is a computer-controlled system that regulates the speed of extension (0.1 mm/s) and measures the loading force (in grams) for each extension point (in millimeters). For circumferential extension analyses, a pair of stainless steel 0.5-mm hooks were threaded into aortic rings and secured to the TA 96 system clamps. For longitudinal extension analyses, aortic segments were secured in the TA 96 system clamps directly.

+/+ mice (B6C3Fe a/a) were purchased to serve as additional control animals, because the *oim* mutation is maintained in the B6C3Fe a/a background.[22] All animals were housed in a facility approved by the American Association for Accreditation of Laboratory Animal Care (AAALAC), provided free access to water and food (standard laboratory diet, autoclavable rodent laboratory chow, 5010, Purina Mills, Richmond, Ind), and handled according to an approved protocol and the approved regulations of the facility. *Oim/oim, oim/+*, and +/+ genotypes were confirmed by means of polymerase chain reaction-restriction fragment length polymorphism analysis.[23] Mice (age, 4-7 months) were euthanized with CO_2 asphyxiation for biomechanical and histologic analyses.

Preparation of the aortic segments. Aortas were harvested with a Weck operating microscope (J. K. Hoppl Corp, Lindenhurst, NY) with a lens magnification of 15×. The chest of the animals was opened via a median sternotomy, and the thoracic aorta was dissected from the surrounding adipose tissue and harvested from the aortic root to the diaphragmatic crurae. For circumferential analyses, two 5-mm long aortic rings were prepared: a proximal ring including aortic root and aortic arch and a distal aortic ring including the descending aorta just below the take off of the left subclavian artery. A pair of 0.5-mm diameter stainless steel hooks was inserted in each aortic ring. For longitudinal analyses, the whole segment of the descending aorta, from the left subclavian artery to the diaphragm, was excised for use in the longitudinal extension analyses. The infrarenal aorta was not analyzed because of its decreased size (< 5 mm in length and < 1 mm in diameter).

Biomechanical studies. To obtain circumferential load-extension curves, we attached each aortic ring to a TA-XT2 texture analyzer (tensile testing apparatus; Texture Technologies, Scarsdale, NY), placed it in a Krebs solution bath at room temperature (24 ± 1°C), and stretched it at a constant tensile speed of 0.1 mm/s (Fig 1). During the aortic ring extension (length [l, in millimeters]), the required tensile load (Force [F, in grams]) was recorded at 0.01-second intervals. The maximal tensile load (F_{max}) is the greatest load (force) before vessel breakage (Fig 1, value indicated by *C*). While the aortic segment is deformed by tensile load, the wall exerts an increasing retractive force, aiming to a final equilibrium. Stiffness of the aortic ring results from the relationship between deformation (extension) and tensile load and can be expressed as:

$$E = f/l$$

in which E is the elastic modulus, f is the tensile load, and l is the extension. Because of the nonlinear relationship, the incremental elastic modulus (IEM = $\Delta f/\Delta l$) was used as a means of comparing the stiffness of the aortic segments among the genotypes. The IEM was calculated to be the ratio of the incremental load (in grams) to the incremental extension (in millimeters). The IEM was estimated by performing a linear regression analysis on each load-extension curve. Best-fit lines were obtained at low and high (1.5× to 2× diameter) degrees of extension in the aortic rings. Elastin bears most of the load at low degrees of extension, whereas collagen bears most of the load at higher degrees. The slope of the regression line at high degrees of extension was used to estimate the IEM, because this is the region in which differences in collagen structure are observed. IEM and F_{max} values were standardized to dry mass weight of the respective aortic sample (in milligrams).

In the longitudinal direction, aortic segments of the whole descending aorta were extended at 0.1 mm/s along the longitudinal axis (axial stretching), and the F_{max} and the IEM were determined. Fig 1 represents schematically the TA-XT2 texture analyzer used for the aortic tissue extension.

Hydroxyproline assay. The hydroxyproline content was used as a means of determining the total amount of collagen in the aortic wall. Hydroxyproline represents 20% to 30% of the amino acids of mature collagen molecules.[4,5] After load-extension curve analyses, the aortic tissue was dried and weighed, and the total hydroxyproline content was determined by means of a colorimetric assay (on the basis of the oxidation of hydroxyproline to a compound that reacts with *p*-dimethylaminobenzaldehyde to form a chromophore) established by Stegemann and Stadler.[24]

Histologic analysis. Aortic sections were harvested for histologic and morphologic analyses from 2 *oim/oim* and 3 +/+ mice. Aortas were perfused at 112 mm Hg, a pressure that mimics the normal *oim/oim* mouse systolic pressure as determined by means of tail-cuff plethysmography. Perfusion was performed through the left ventricle

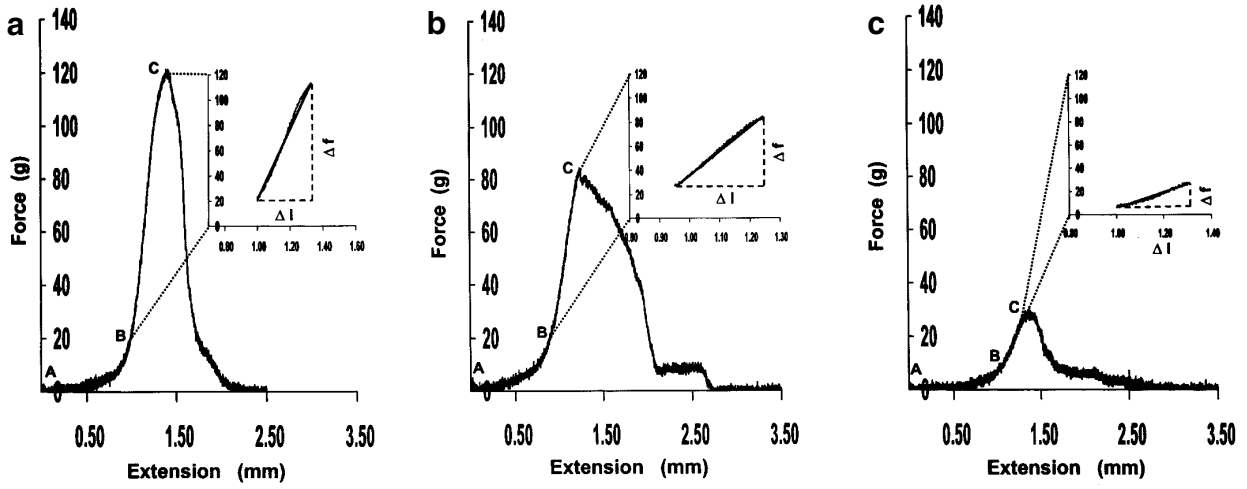

Fig 2. Representative circumferential load–extension curves obtained from the descending aortic rings of wildtype (+/+; **a**), heterozygous (*oim/+*; **b**), and homozygous (*oim/oim*; **c**) mice. The *AB* segment represents low-degree extension, and the *BC* segment represents high-degree extension (1.5-2 × initial aortic diameter). Inset is linear analysis and best-fit line of segment *BC*. Incremental elastic modulus was calculated from slope of *BC* segment, IEM = df/dl.

with 0.9% NaCl (2 minutes), followed by neutral buffered 10% formalin fixation (3 minutes). Aortas were then harvested and processed for histologic examination. Transverse sections were collected from the aortic arch, proximal descending thoracic aorta, and the proximal abdominal aorta. Five-micron sections of each aorta were stained with hematoxylin and eosin as a means of qualitatively assessing cell number and morphology, picrosirius red as a means of assessing collagen, and Verhoeff-van Gieson as a means of assessing elastin.

Statistical analysis. Differences in F_{max} and IEM between *oim/oim*, *oim/+*, and +/+ mice were evaluated by means of analysis of variance and analysis of covariance, with age as the covariant. Statistical significance was defined as a *P* value less than .05.

RESULTS

Six *oim/oim*, 8 *oim/+*, and 6 +/+ mice were sacrificed for the circumferential analyses. The mean body weights of the +/+, *oim/+*, and *oim/oim* mice were 25.0 ± 2.1 g, 26.3 ± 2.6 g, and 20.8 ± 2.1 g, respectively. Although *oim/oim* mice weighed 21% and 17% less than the *oim/+* ($P = .001$) and +/+ ($P = .02$) mice, there appeared to be no differences in aortic diameters or lengths.

Biomechanical studies. Representative circumferential load extension curves for the descending aortic rings are depicted in Fig 2. Complete disruption of vessels was noted at ring extensions that were 1.5 to 2 times the initial diameters for all genotypes (indicated by the F_{max} value of *C*). As demonstrated by means of Fig 2, the curves were nonlinear. At low degrees of stretch, small load increments resulted in large-size deformation (segment *AB* in the curve). At high degrees of ring extension (1.3 to 2 times the diameter of the ring) in which collagen bears most of the load, the same load increments resulted in much smaller deformation (segment *BC*). Linear regression analysis of segment BC lead to the best-fit line (inset of Fig 2, *a*, *b*, and *c*), which was used as a means of estimating the IEM.

Fig 3 summarizes the results from the load-extension curves obtained from circumferential extension of the ascending aortas. Circumferentially, the maximum tensile load (F_{max}) of the *oim/oim* ascending aorta (132 ± 42 g/mg) was significantly reduced, as compared with the F_{max} of the +/+ ascending aorta (219 ± 75 g/mg, $P = .04$). The *oim/+* mice demonstrated intermediate values for F_{max} (185 ± 66 g/mg), although the results were not significantly different from the *oim/oim* or +/+ groups (Fig 3, *a*). When the stiffness of the ascending aorta was evaluated, the IEM values of *oim/oim* (IEM = 379 ± 332 g/mm/mg), *oim/+* (IEM = 309 ± 126 g/mm/mg), and +/+ (IEM = 417 ± 244 g/mm/mg) mice were not statistically different. However, there was a trend for the *oim/oim* and *oim/+* aortas to be more extensible (lower IEM values) than the +/+ ascending aortas (Fig 3, *b*).

In contrast, circumferential extension of the descending aortas demonstrated significant differences in both the breaking strength and stiffness between *oim/oim*, *oim/+*, and +/+ mice (Fig 4). The maximal load (F_{max}) required to circumferentially disrupt the descending aortic ring of +/+ mice was 447 ± 124 g/mg, whereas the F_{max} of *oim/+* and *oim/oim* mice descending thoracic aortas were 242 ± 112 g/mg ($P = .002$) and 103 ± 42 g/mg ($P = .001$), respectively (Fig 4, *a*). Similarly, the +/+ mice had stiffer (less extensible) distal aortic rings (IEM, 788 ± 128 g/mm/mg) than the *oim/+* (486 ± 271 g/mm/mg, $P = .01$) and *oim/oim* (188 ± 70 g/mm/mg, $P = .0001$) mice (Fig 4, *b*).

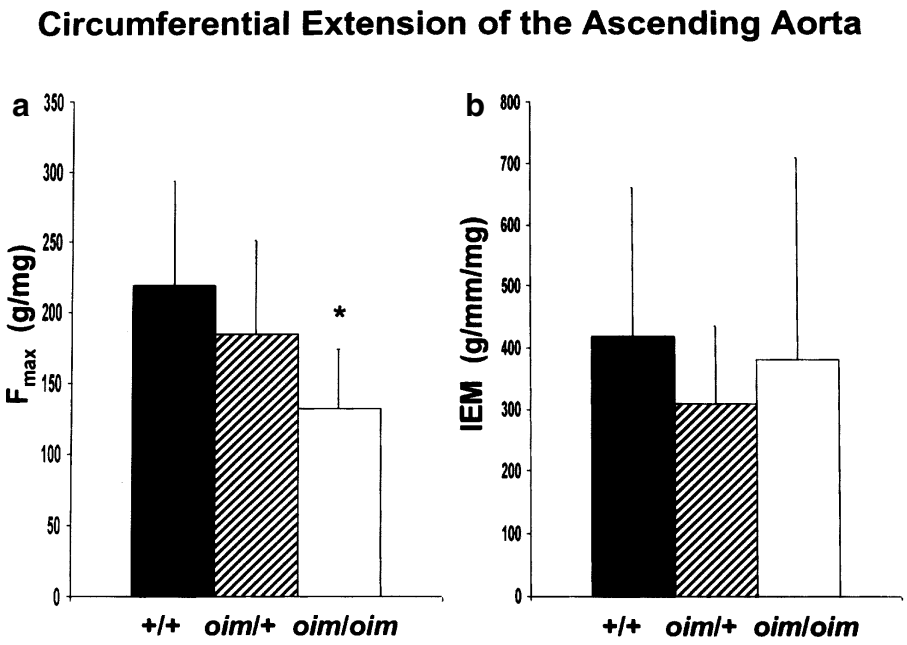

Fig 3. Analyses of circumferential maximum breaking strength (F_{max}; **a**) and incremental elastic modulus (*IEM*; **b**) of the ascending aorta of wildtype (+/+; n = 6; *black bar*), heterozygous (*oim/+*; n = 8; *diagonal bar*), and homozygous (*oim/oim*; n = 6; *open bar*) mice. Values are expressed as the mean plus or minus SD in grams (F_{max}) and grams per millimeter (*IEM*) per milligram of dried aortic tissue. $*P \leq .05$.

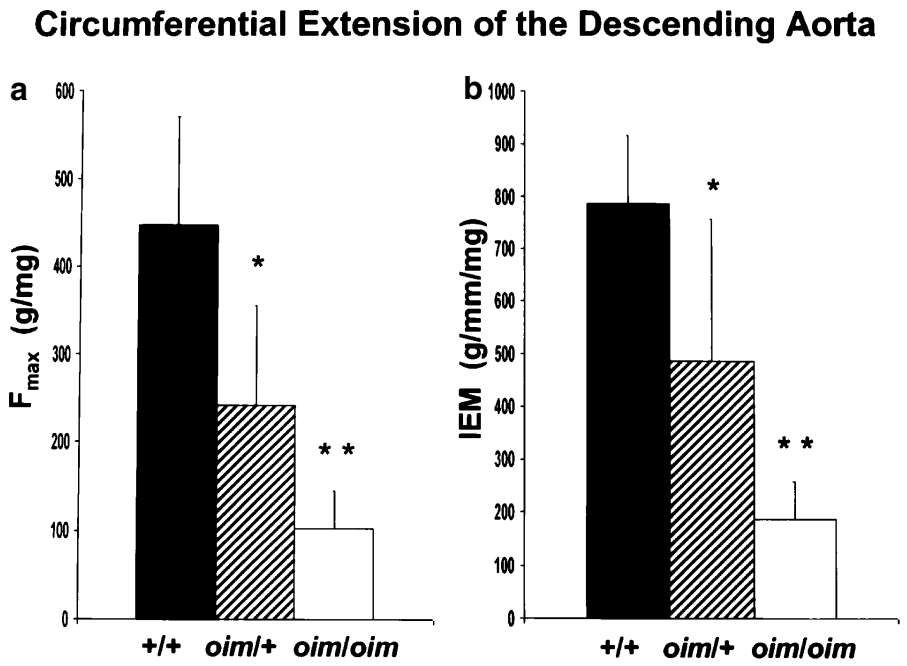

Fig 4. Analyses of circumferential maximum breaking strength (F_{max}; **a**) and incremental elastic modulus (*IEM*; **b**) of descending aorta of wildtype (+/+; n = 6; *black bar*), heterozygous (*oim/+*; n = 8; *diagonal bar*), and homozygous (*oim/oim*; n = 6; *open bar*) mice. Values are expressed as mean ± SD in grams (F_{max}) and grams per millimeter (*IEM*) per milligram of dried aortic tissue. $*P \leq .01$; $**P \leq .001$.

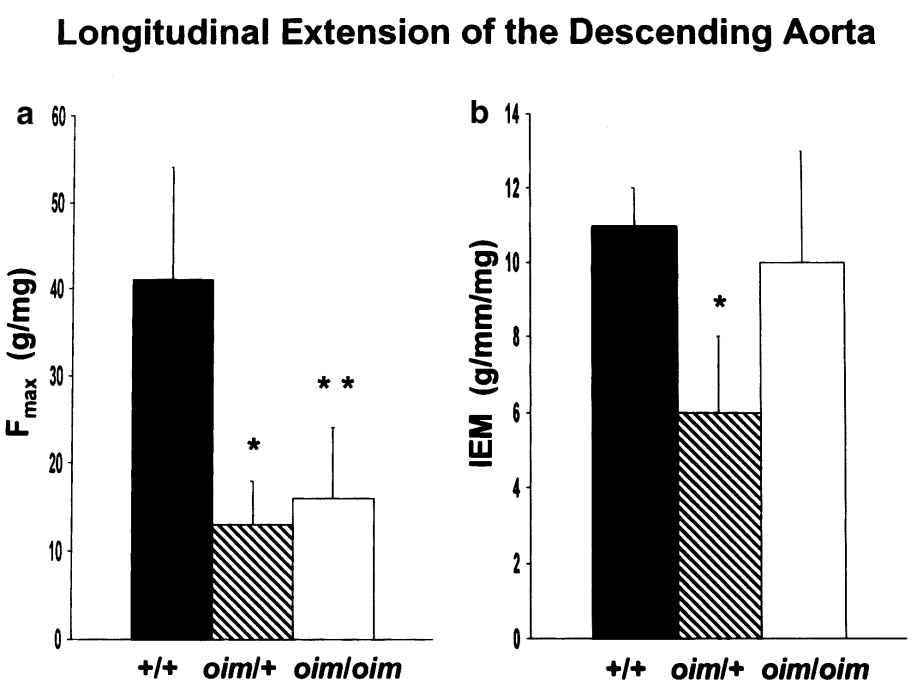

Fig 5. Analyses of longitudinal maximum breaking strength (F_{max}; **a**) and incremental elastic modulus (*IEM*; **b**) of descending aorta of wildtype (+/+; n = 7; *black bar*), heterozygous (*oim/+*; n = 3; *diagonal bar*), and homozygous (*oim/oim*; n = 5; *open bar*) mice. Values are expressed as mean ± SD in grams (F_{max}) and grams per millimeter (*IEM*) per milligram of dried aortic tissue. $*P \leq .05$; $**P = .003$.

Longitudinal extension studies of the descending thoracic aortas were performed in 5 *oim/oim*, 3 *oim/+*, and 7 +/+ mice. The F_{max} of the +/+ mice (41.7 ± 13.5 g/mm/mg) was significantly greater than that of the *oim/oim* (16.4 ± 8.5 g/mg, $P = .003$) and *oim/+* mice (13.11 ± 5.2 g/mg, $P = .017$; Fig 5, *a*). However, no significant difference was noted in the longitudinal extensibility (IEM) of the +/+ descending aorta (10.7 ± 1.4 g/mm/mg), as compared with the *oim/oim* descending aorta (9.8 ± 3.3 g/mm/mg, $P = .512$; Fig 5, *b*). The IEM of the *oim/+* mice (5.9 ± 1.6 g/mm/mg) did appear different from that of the +/+ mice ($P = .034$), but the real significance of this is unclear and may reflect, in part, the small sample number of *oim/+* mice (n = 3).

Hydroxyproline assay. Hydroxyproline content (a measure of total collagen) was measured in 6 +/+, 3 *oim/+*, and 4 *oim/oim* mice. There were no significant differences in the amount of total collagen present in the descending thoracic aortas of the +/+ (13.5 ± 3.8 μg collagen/mg dry tissue), *oim/+* (12.0 ± 1.6 μg/mg), and *oim/oim* (12.3 ± 3.0 μg/mg) mice. This suggests that the altered biomechanical properties may not simply reflect quantitative differences in total collagen, but more qualitative alterations in the fibrillar organization and architecture.

Histologic analysis. Preliminary histologic evaluation of the descending thoracic aorta was performed in 2 *oim/oim* and 3 +/+ mice (Fig 6). Although only a limited number of animals were examined histologically, examination of hematoxylin and eosin–stained sections suggested there were no lesions or major differences in cell numbers. Furthermore, there appeared to be no major differences in aortic wall thickness or diameter between *oim/oim* and +/+ mouse aortas. In addition, there were no obvious histologic abnormalities or differences in the lamellar or elastin organization between *oim/oim* and +/+ mouse aortas.

DISCUSSION

We present evidence that the thoracic aorta of the *oim/oim* mouse exhibits decreased breaking strength and greater extensibility in the absence of proα2(I) collagen chains and/or presence of the homotrimeric type I collagen isotype. The most pronounced biomechanical differences were seen with circumferential extension of the descending aorta. The average breaking strength (F_{max}) circumferentially in the descending *oim/oim* mouse aorta was only 23% of the F_{max} in +/+ mouse aorta. Similarly, the *oim/oim* mouse descending aorta had an IEM that was 24% of the value in +/+ mouse aorta. The differences were not as dramatic during circumferential extension of the ascending aorta. Overall, the circumferential F_{max} of the ascending aorta (range, 132 to 219 g/mg) was much lower than the F_{max} of the descending aorta (range, 242-

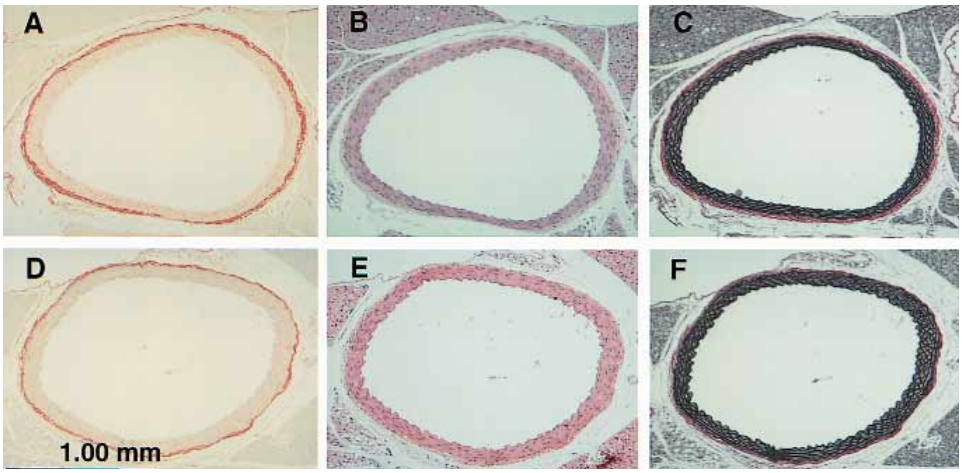

Fig 6. Histology of media thoracic aortic sections of 7-month-old wildtype (+/+; **A, B, C**) and homozygous (*oim/oim*; **D, E, F**) mice stained with picrosirius red **(A, D)**, hematoxylin and eosin **(B, E)**, and Verhoeff-van Gieson (**C, F**; original magnification, 10×).

447 g/mg). The circumferential F_{max} of the ascending *oim/oim* aorta was approximately 60% of the value in the +/+ aorta. These findings are consistent with our understanding that collagen and elastin bear the majority of the wall stress and determine the stiffness (compliance) of the aorta.[2] With increasing distance of the vessel from the heart, the elastin content is known to decrease and the collagen content to increase, with a net result of higher collagen-to-elastin ratios.[18,25]

The aorta is an "elastic artery," and subsequently its media is composed of layers of smooth muscle cells interspersed with clearly defined lamellae of connective tissue. These lamellar units, composed predominantly of elastin and collagen lying in parallel, represent the functional and structural unit of the aortic wall that responds to pressure and tangential stress and are responsible for the passive mechanical properties of the vessel.[1,2] Several studies suggest that elastin determines the aortic compliance at low physiologic pressures and that collagen progressively takes over load-bearing at higher pressures.[2,26-28] Accordingly, aortic stiffness also progressively increases from the ascending and descending thoracic aorta to the abdominal aorta.[18]

In addition, the aortic wall is anisotropic, having different mechanical properties in the three orthogonal directions, circumferential, longitudinal, and radial.[2] Enzymatic degradation studies demonstrated that elastin bears the load in all three directions, whereas collagen bears the load almost exclusively in the circumferential direction, and smooth muscle cells do not significantly contribute directly to the passive "static" biomechanical properties of the aortic wall.[25,29-32] During the longitudinal stretching, all the aortic samples, regardless of mouse genotype, demonstrated much lower breaking strength (range, 13 to 42 g/mg) and higher extensibility (range, 6-11 g/mm/mg) than when they were stretched circumferentially. These findings are also consistent with the anisotropic nature of the aortic wall. Moreover, the longitudinal extension showed no differences in the stiffness of the aortas in *oim/oim* and +/+ mice, which is also consistent with the decreased role of collagen along the longitudinal axis. However, to our surprise, there was still a significant difference in F_{max} longitudinally between the genotypes, suggesting that type I collagen does influence indirectly or directly the biomechanical properties along the longitudinal axis. One can easily postulate that deposits of the homotrimeric type I collagen isotype and/or the absence of the proα2(I) collagen chain may alter the fibrillar structure and architecture of the collagen fibrils and influence the structure and function of the other components of the lamellar units.

In addition, we found no significant differences in total collagen content or the morphological structure/organization of the aortas of *oim/oim* and +/+ mice, suggesting that the absolute amount of collagen is not the only factor that determines arterial wall strength and further suggesting that the ultrastructural collagen composition and organization of the aortic wall must play a significant role.

It is quite likely that all three components, elastin and types I and III collagen, contribute to the ultimate structural integrity of the aorta. We postulate that a structural defect in any one or more of these components increases the load-bearing on the other components of the aortic wall and, thus, makes the vascular wall more susceptible to fatigue and failure. There is a great deal of clinical evidence from the pathogenesis of certain connective tissue disorders that supports this hypothesis. EDS types IV and VI result from defects affecting type III and I collagens, respectively, and are strongly associated with decreased arterial wall integrity and cardiovascular deficits, including

mitral valve prolapse, multiple arterial ruptures, and aortic dissections.[19-21,33] Two unrelated individuals with classical EDS (EDS type I/II) who have been previously described synthesize only homotrimeric type I collagen and are biochemically similar to the *oim* mouse[22] and certain recessive forms of human type III OI.[15,16] Both individuals had cardiovascular complications, a grade IV aortic regurgitation and a severe mitral valve prolapse.[12-14] However, no data were presented about the integrity of their aortas. The significance of our findings with the *oim* mouse aortas in the clinical course of patients with aortic aneurysmal disease or with type I collagen deficiencies is yet to be determined. The cardiovascular complications of OI, particularly in relation to aortic diseases, have not been well characterized. Only a handful of cases of spontaneous aortic dissection directly related to OI are reported in the literature.[34-37] Vetter evaluated cardiovascular function in 58 patients with OI types I, III, and IV and determined that patients with OI type III were more likely to have ectatic aortas.[38] It is difficult to make any direct clinical correlation with the *oim/oim* mouse, although it is clinically most similar to OI type III, because its molecular defect is relatively rare (autosomal recessive inheritance of a functionally null COL1A2 gene), with only a few reported human cases.[15,16]

Deficiencies in elastin and collagen have also been implicated in the pathogenesis of aneurysmal degeneration in patients who do not have Marfan syndrome or EDS.[2,17,39-41] Although there is a well-demonstrated correlation between elastin decrease and aneurysm formation, there still remains a lot of confusion regarding the role of collagen and the aneurysmal aorta in the literature; collagen concentrations have been found to be increased in some studies and decreased or unchanged in others.[2,17,39,40,42,43]

This study demonstrates the biomechanical consequences of exclusive expression of homotrimeric type I collagen on the thoracic aorta of the *oim/oim* mouse and represents a unique model for investigating the role of the proα2(I) collagen chain in the structure and function of the vasculature and for defining the mechanisms and tissue-specific adaptations to over-expression of the homotrimeric isotype of type I collagen. The *oim* mouse provides a potential paradox for cardiovascular disease and aging. The increased extensibility of the *oim/oim* aorta may protect the *oim/oim* mouse against the development of elevated arterial pressures, but the *oim/oim* aorta with its significantly reduced breaking strength may also be significantly compromised when subjected to elevated arterial pressures or other vascular stressors. Long-term follow-up of these mice during aging and under cardiovascular stress, by using serial ultrasonic imaging of their aortas, analysis of the aortic velocity profile under different conditions of systemic blood pressure, and analysis of the ultrastructure of the collagen should significantly contribute to our understanding of the role of the extracellular matrix architecture in vascular function and disease. Finally, the greatest significance of the *oim* mouse model is that it provides a foundation for future studies. In conjunction with other cardiovascular mouse models, such as the elastin-deficient mouse,[44] it will allow for very defined breeding strategies, making it finally possible to systematically evaluate the role of multigene interactions in vascular and aneurysmal disease.

We thank Russell Stevens for his excellent technical assistance in preparation of aortic samples.

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Submitted Jul 4, 2000; accepted Dec 5, 2000.