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Collagen alteration in vascular remodeling by hemodynamic factors

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Abstract The collagen alterations in the vascular wall remodeled by hemodynamic change were investigated by electron microscopy and immunohistochemistry. The left anterior descending coronary artery (LAD) without a myocardial bridge (MB) showed both lower matrix metalloproteinase-1 (MMP-1) expression and a smaller extent of spiraled collagen (SC) distribution than the LAD wall with MB, in which the intima was influenced by high shear stress. In the wall of the varicose great saphenous vein (GSV) the expression of MMP-1 was lower, while the expression of prolyl 4-hydroxylase was higher, than in the normal GSV. The extent of SC distribution in the intima and media of the varicose GSV was smaller than that in the normal GSV. An analogous difference in results was demonstrated between the portal vein (PV) of patients with liver cirrhosis and normal PV. However, the levels of expression of MMP-2, MMP-9 and tissue inhibitors of MMP (TIMPs) in these pathologic vessels were not different from those in the corresponding normal vessels. The results indicate that hemodynamic forces such as shear stress and increased intravascular blood pressure contribute to the collagen alterations in the vascular wall, which may lead to vascular wall remodeling.

Key words Hemodynamic factor · Human blood vessel · Matrix metalloproteinases · Spiraled collagen · Collagen metabolism

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Introduction

The migration and proliferation of smooth muscle cells (SMCs) and the metabolism of the extracellular matrix (ECM) lead to vascular remodeling, in which SMCs are the main cellular component and the ECM is a dynamic structural element [2, 7, 21]. SMCs synthesize the major ECM components of the vascular wall, such as collagen types I, III, IV and V, proteoglycans, and elastin [7]. They also synthesize and secrete not only matrix metalloproteinases (MMPs) but also tissue inhibitors of MMPs (TIMPs), as do vascular endothelial cells [6, 8, 11, 17]. In the MMP family, which consists of various MMPs, MMP-1 (interstitial collagenase) degrades native collagens types I, II and III, while MMP-2 (gelatinase A) and MMP-9 (gelatinase B) have the specific ability to degrade denatured fibrillar collagens (gelatin) and native collagen type IV [7]. The degradation of vascular collagen by MMPs also influences SMC proliferation and migration from the media into the intima [2, 23, 29]. On the other hand, TIMP-1 and TIMP-2, natural inhibitors of MMPs, have important roles in supporting the proteolytic/antiproteolytic balance [7]. The overexpression of TIMP-1 inhibits the proliferation and migration of vascular SMCs, resulting in subsequent inhibition of intimal hyperplasia [3, 9]. These findings on the independent roles of MMPs and TIMPs imply that vascular remodeling, and especially intimal remodeling, is highly dependent on the functions of SMCs and endothelial cells.

Vascular remodeling takes place in response to hemodynamic changes and vascular wall diseases [7]. Hemodynamic factors such as low shear stress acting on the arterial intima are implicated in the susceptibility to atherosclerotic lesions [27, 42], as is an increase in transmural pressure (hypertension), which results in intimal fibrocellular thickening [36]. As just one such example, the myocardial bridge (MB), which frequently covers a part of the course of the left anterior descending coronary artery (LAD), running in the epicardial adipose tissue for almost its whole length, surprisingly regulates the hemodynamic factors [19]. The LAD intima beneath a

MB, which is subject to a high shear stress compared with the intima of the nonbridged part, is always exempt from the development of atherosclerosis [19, 20]. While collagen fibrils and elastin are the contributors to the tensile strength of the blood vessel wall at physiological pressures, hemodynamic alteration affects the morphological changes in collagen fibrils [38]. Spiraled collagen (SC), which is a specific form of defrayed collagen fibril showing spiral thickening with a periodic banding pattern [13], has been demonstrated in vascular tissue under various conditions [14, 30, 35, 37, 38]. Furthermore, in our previous study it was demonstrated more frequently in the LAD beneath an MB than that in the nonbridged LAD, and the expression of MMP-1 was conspicuous in SMCs and endothelial cells in the LAD beneath an MB [18]. SC was also abundant in normal veins in which there were high levels of MMP-1 [18]. These findings on SC distribution and MMP-1 expression indicated that SC might be preferentially formed under conditions of physiological degradation of normal collagen fibrils in the normal blood vessels [18]. Thus, hemodynamic change that stresses the vascular intima may have a direct or indirect influence on the collagen metabolism in the vascular wall.

In the present study, we investigated the expression of MMPs, TIMPs and prolyl-4-hydroxylase in the blood vessels by immunohistochemistry, and the distribution of SC by electron microscopy. We discuss the relationship between hemodynamic change and vascular remodeling in the LADs with and without MB, and between increased transmural pressure and vascular remodeling in both the great saphenous veins (GSVs) and the portal veins (PVs).

Materials and methods

The LADs (n=25), GSVs (n=28) and PVs (n=23) were obtained from consecutive autopsied or surgical cases. The tissues were the generous gifts of patients or patients' relatives, with documentation of the donors' agreement to their use in this study. The LADs were dissected from 25 consecutive autopsied cases and divided into two groups: LADs beneath MB {[LAD MB(+) group], n=10, 7 male and 3 female, mean age 56.4±7.7} and LADs without MB {[LAD MB(–) group], n=15, 13 male and 2 female, mean age 63.2±9.11}, respectively. The normal GSVs were also obtained from 10 autopsied cases [(control GSV group), n=10, 5 male and 5 female, mean age 69.2±11.2] and the varicose GSVs from consecutive surgical cases [(varicose GSV group), n=18, 6 male and 12 female, mean age 56.5±5.9]. The PVs were obtained from autopsied cases by dissection from the extrahepatic portal trunk. They were divided into two groups: PVs from cases without any hepatic lesion [(control PV group), n=10, 7 male and 13 female, mean age 66.1±19.2] and PVs from cases with liver cirrhosis, which had been subject to portal hypertension [(LCPV group), n=13, 11 male and 2 female, mean age 59.8±8.6], respectively. The GSVs were cut transversely in the flow axis, and the thickened walls of varicose GSVs were used. The LADs and PVs were cut longitudinally.

Light microscopic examination

The materials were fixed with neutral buffered formalin and then embedded in paraffin. Thin sections were treated with hematoxylin–eosin and elastic van Gieson's stainings. In addition, mouse monoclonal antibodies against human MMP-1 (Oncogene Science, Mass.), MMP-2, MMP-9, TIMP-1, TIMP-2, prolyl-4-hydroxylase (Fuji Chemical, Toyama, Japan) and human alpha smooth muscle actin (DAKO, Glostrup, Denmark) were used for immunohistochemistry. Immunohistochemistry for MMPs, TIMP-2, prolyl-4-hydroxylase and alpha smooth muscle actin was performed by the labeled streptavidin–biotinylated peroxidase complex method (Dako, Carpinteria, Calif.). Tissues for immunohistochemistry, except for those treated with the antibody against human alpha smooth muscle actin, were pretreated with 5% urea in Tris-buffered saline. Immunohistochemistry for TIMP-1 was performed using the catalyzed signal amplification system (Dako, Carpinteria). For negative controls, primary antibodies were replaced with normal mouse IgG. Slides were counterstained with hematoxylin.

From the results of immunohistochernistry using antibodies against MMPs, TIMPs and prolyl-4-hydroxylase, the extents of positive findings in the intima and media were graded in five categories according to the amounts of immunopositive cells or area: grade 0, negative result; grade 1, scattered positive cells (less than 5%) or mild positive spots in the stroma; grade 2, small proportion of positive cells (6–30%) or moderate positive spots in the stroma, grade 3: moderate amount of positive cells or positive stromal finding (from grade 2 to grade 4); and grade 4, diffusely scattered positive cells (more than 50%) or diffusely scattered positive spots in the stroma, respectively. In each group of the six kinds of blood vessels, the number of cases in each category was determined. Statistical comparison between the immunohistochemical evaluations of the LAD MB(+) and LAD MB(–) groups was performed by the Mann-Whitney U test. The same statistical analysis was employed to compare the control GSV and varicose GSV groups and the control PV and LCPV groups.

Electron microscopic examination

The materials as mentioned above were fixed with glutaraldehyde and osmium tetroxide and were embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate, silicotungstic acid and lead citrate, and then observed with a JEM 1200EX-II electron microscope (JEOL, Tokyo, Japan). Each section was observed at 30,000-fold magnification, and the distribution of SC was investigated in 40 fields arbitrarily selected from the intima and media. The extent of SC distribution was graded into four categories according to the number of SC-containing fields: grade 0, absent; grade 1, fewer than 8 fields; grade 2, from 8 to 32 fields; and grade 3, more than 32 fields. The extent of SC distribution in the LAD MB(+) and LAD MB(–) groups was compared using statistical analysis by the Mann-Whitney U test. In addition, the same statistical analysis was performed to compare the SC distribution in the control GSV and varicose GSV groups and the control PV and LCPV groups.

Results

Light microscopy

All 15 cases in the LAD MB(–) group had intimal lesions: 1 case with fatty streak, 12 with atheroma, and 2 with severe fibrocellular intimal thickening. In contrast, the 10 cases in the LAD MB(+) group lacked atherosclerotic lesions: 7 cases showed mild fibrocellular intimal thickening, and 3 cases showed neither intimal thickening nor atherosclerotic lesions. The control GSVs had normal venous structures, but all the varicose GSV group showed fibrocellular intimal thickening and medial thickening. The intima of the LCPVs also revealed fi-

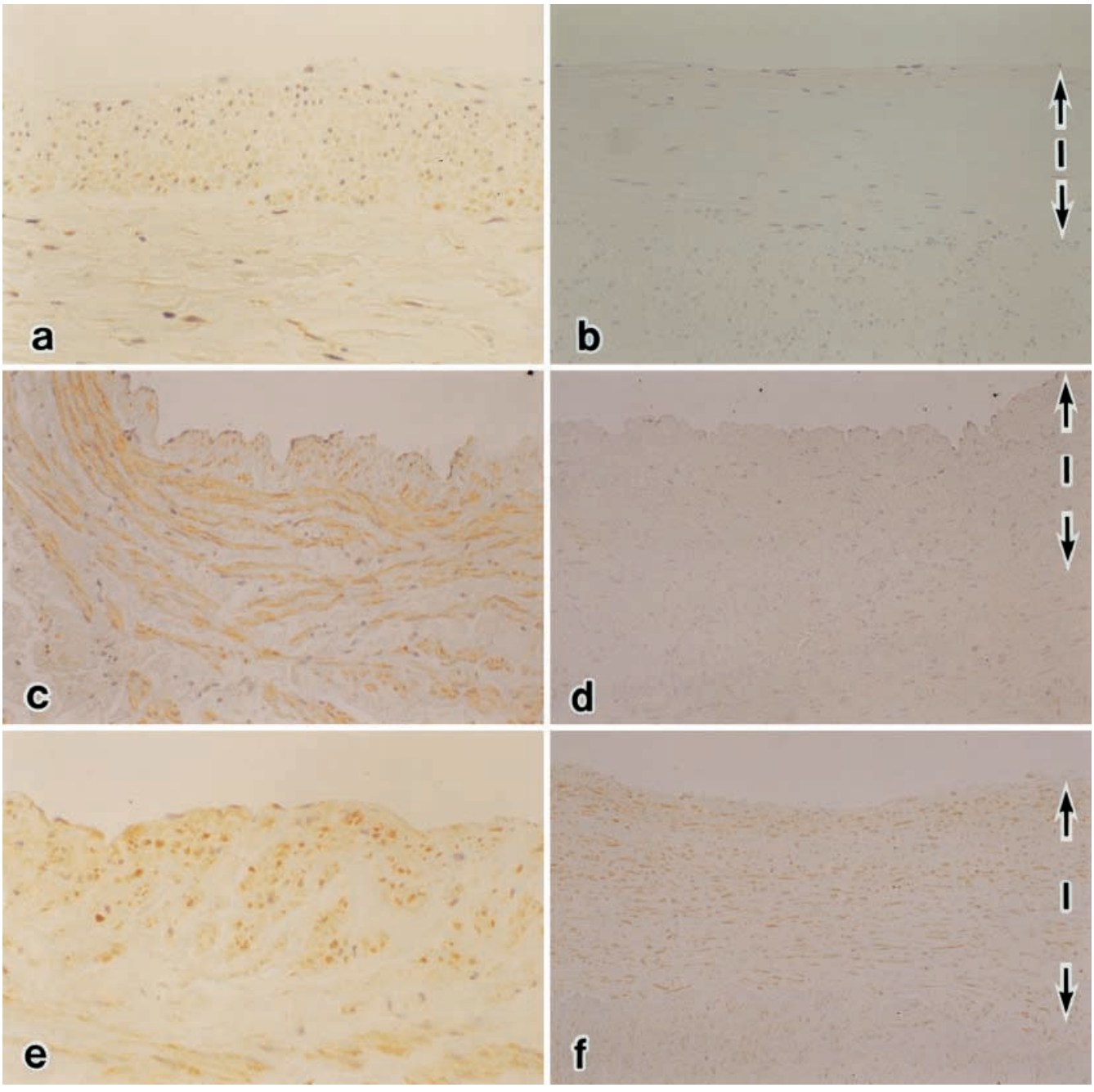

Fig. 1 Immunohistochemistry for MMP-1 in **a**, **b** LADs, **c**, **d** GSVs, and **e**, **f** PVs. **a** In a LAD MB(+) artery without intimal lesion, MMP-1 is diffusely detected in the cells of the arterial wall. ×150 **b** In contrast, immunostaining of MMP-1 is negative in a LAD MB(–) wall with fibrocellular intimal thickening (*I* intima). ×134 **c** MMP-1 is also positive in the intimal and medial cells of a control GSV. ×134 **d** In a varicose GSV with severe intimal fibromuscular thickening, MMP-I is not found. ×88 **e** A control PV reveals extensive positive immunoreactivity for MMP-1. ×150) **f** An LCPV shows marked intimal thickening in which MMP-1 is positive in the intimal and medial cells. ×88

brocellular thickening, while the control PVs maintained normal intima. The blood vessels of the LAD MB(–), varicose GSV and LCPV groups were thus regarded as morbid blood vessels, in contrast to the blood vessels in their normal counterparts, such as the LAD MB(+), control GSV and control PV groups.

The results of immunohistochemistry are summarized in Tables 1 and 2. In all cases of the LAD MB(+) group, MMP-1 was diffusely present in the endothelial cells, intimal cells and medial SMCs, accompanied by positive spots in the stroma (Fig. 1a), regardless of the presence of diffuse intimal thickening. Although the 6 cases in the LAD MB(–) group showed moderate to strong immunoreactivity against MMP-1, positive immunoreactivity

against MMP-1 was not found or was mild in the 9 cases in the LAD MB(–) group. The extent of positive immunoreactivity for MMP-1 in the LAD MB(+) group was significantly greater than that in the LAD MB(–) group (Table 1). In the GSVs and PVs, MMP-1 was also positive in the cytoplasm of endothelial cells, intimal cells and medial SMCs, except for 5 cases of the varicose GSV group (Fig. 1c–f). MMP-1 expression was more extensive in the varicose GSV and LCPV groups than in their normal counterparts (Table 1).

MMP-2 expression was observed in the cytoplasm of intimal cells, medial SMCs and endothelial cells, and sometimes in the stroma, of the LADs except for in 10 cases. Weak MMP-9 expression was observed in the intimal and medial stroma of only 5 cases of the LAD MB(–) group, 19 of the GSVs and 7 of the PVs, but was not observed at all in the LAD MB(+) group. While 33 of the LADs, GSVs and PVs showed negative immunoreactivity against TIMP-1, the other 43 cases showed positive immunoreactivity against TIMP-1 on endothelial cells and SMCs. TIMP-2 was observed in endothelial cells and SMCs in all of the GSVs and PVs, as well as in 18 of the LADs. Regarding to the extent of immunoreactivity against MMP-2, MMP-9 and TIMPs in the blood vessel walls, there was no significant difference between normal vessels and their pathologic counterparts, as shown in Table 2.

Prolyl-4-hydroxylase was detected in the intimal cells, endothelial cells, and SMCs in the media. The extent of positive immunoreactivity in the LAD varied regardless of the presence of MB (Fig. 2). There was no significant difference in the expression of prolyl-4-hydroxylase between the LAD MB(–) and LAD MB(+) groups. Prolyl-4-hydroxylase was negative in the control GSV and control PV groups, except for in 6 cases. However, it was positive in 26 cases (84%) of the varicose GSVs and LCPVs. The extent of immunoreactivity for prolyl-4-hydroxylase in the varicose GSV and LCPV groups was significantly greater than that in their normal counterparts (Table 2).

Electron microscopic observations

Among the 10 cases in the LAD MB(+) group, 7 showed mild fibrocellular intimal thickening without foam cells, while 3 cases had no intimal lesion. SC was recognized in the walls of all cases in the LAD MB(+) group, especially in the intima (Fig. 3a–c). On the other hand, all 15 cases in the LAD MB(–) group had atherosclerotic lesions or fibromuscular intimal thickening to a moderate to marked extent. The thickened intima of the LAD MB(–) group mainly contained abundant collagen fibrils and many migrated SMCs, in addition to a few monocytic foam cells (Fig. 3d–f). No SC was observed in the intima or media of 11 cases in the LAD MB(–) group, and only a moderate amount of SC was found in 4 cases in the LAD MB(–) group. The extent of SC in the LAD MB(+) group was significantly larger than that in the LAD MB(–) group (Table 3).

Table 1 Number of cases with each grade of matrix metalloproteinase (MMP)-1 expression in the six groups of blood vessels (*GSV* great saphenous vein, *LAD* left anterior descending artery, *LC* liver cirrhosis, *MB* myocardial bridge, *PV* portal vein)

Grade	LAD		GSV		PV	
	MB(+) *n*=10	MB(–) *n*=15	Control *n*=10	Varicose *n*=18	Control *n*=10	LC *n*=13
4	7	3	3	0	8	5
3	2	1	2	3	2	3
2	1	2	4	4	0	3
1	0	5	1	6	0	2
0	0	4	0	5	0	0
Significance[a]	*P*<0.05		*P*<0.05		*P*<0.05	

[a] Analyzed by Mann-Whitney's U test

Table 2 The extent of the expression of MMP-2, -9, TIMP-1, -2 and prolyl 4-hydroxylase[a] (*TIMP* tissue inhibitor of MMPs, *NS* not significant)

	LAD		GSV		PV	
	MB(+) *n*=10	MB(–) *n*=15	Control *n*=10	Varicose *n*=18	Control *n*=10	LC *n*=13
Mean score for						
MMP-2	1.10	1.00	2.50	2.61	2.50	2.92
	NS		NS		NS	
MMP-9	0.10	0.40	0.60	0.94	0.30	0.38
	NS		NS		NS	
TIMP-1	1.00	0.60	0.90	0.44	0.80	1.46
	NS		NS		NS	
TIMP-2	1.00	1.67	2.80	2.61	3.90	3.85
	NS		NS		NS	
Prolyl 4-hydroxylase	1.80	1.40	0.20	1.22	0.50	2.62
	NS		*P*<0.01		*P*<0.01	

[a] Significance of differences was analyzed by Mann-Whitney U test. The mean score of each immunoreactivity was derived as follows: the sum of the products obtained by multiplying of each score by the number of cases belonging to each grading category as shown in Table 1 was divided by the total number of cases. Raw data for Mann-Whitney U test are omitted from this table

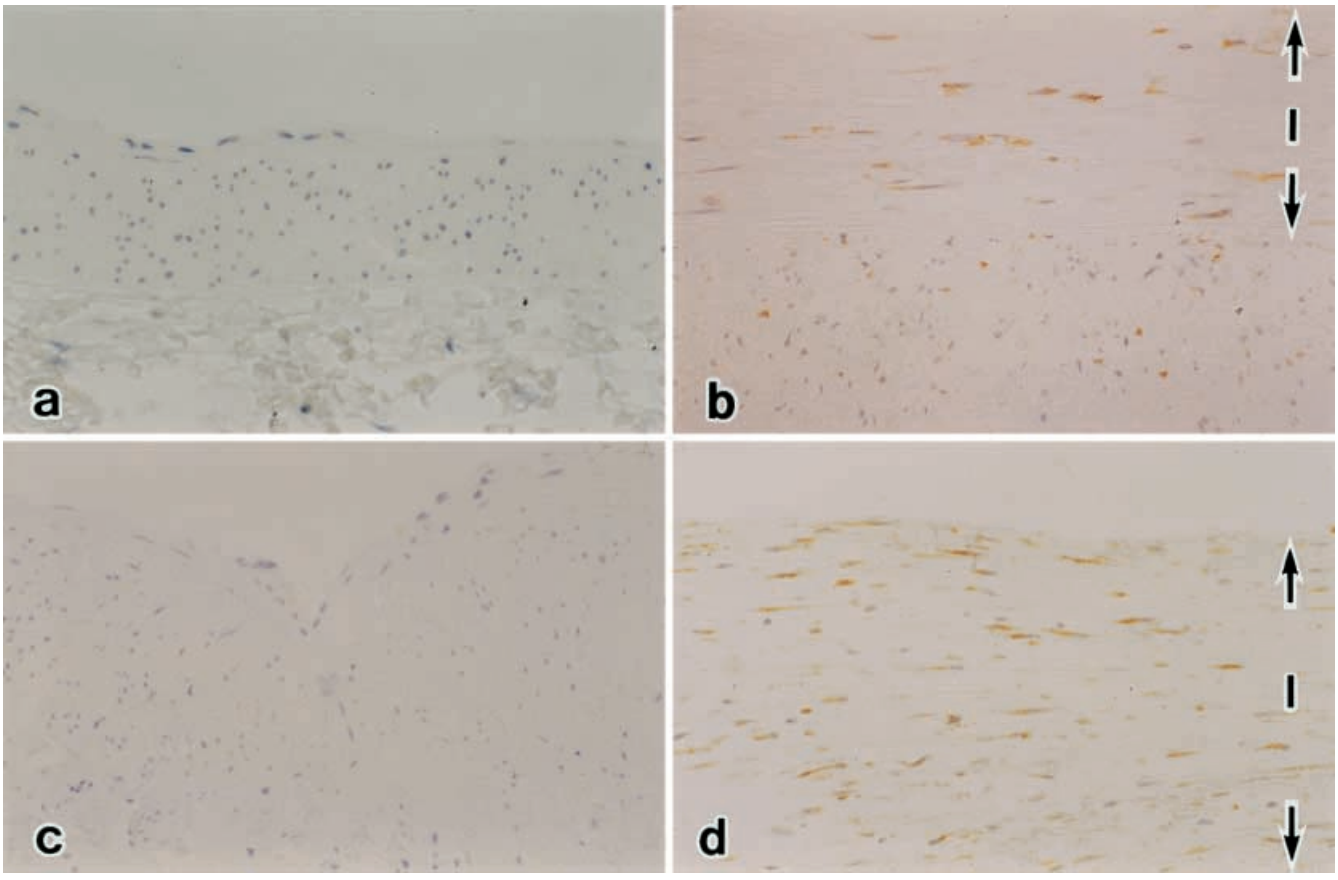

Fig. 2 Immunohistochemistry for prolyl 4-hydroxylase in **a**, **b** the LADs and **c**, **d** the PVs. **a** A LAD MB(+) without intimal lesion shows no expression of prolyl 4-hydroxylase in the arterial wall. ×150 **b** In a LAD MB(–) wall, cells positive for prolyl 4-hydroxylase are scattered in the intima and media (*I* intima). ×110 **c** Immunoreactivity for prolyl 4-hydroxylase is negative in a control PV wall. ×150 **d** In the thickened intima of PVLC, spindle-shaped cells are positive for prolyl 4-hydroxylase. ×210

Table 3 Extent of spiraled collagen in each group of blood vessels (*LC* affected by liver cirrhosis)

Grade	LAD		GSV		PV	
	MB(+) *n*=10	MB(–) *n*=15	Control *n*=10	Varicose *n*=18	Control *n*=10	LC *n*=13
3	5	0	2	0	6	3
2	5	3	4	2	2	5
1	0	1	4	3	1	4
0	0	11	0	13	1	1
Significance[a]	*P*<0.01		*P*<0.01		NS	

[a] Significance was analyzed by Mann-Whitney's U test

The control GSVs showed no intimal lesions and contained normal amounts of elastic fibers regularly arranged in the media. SC was not found in the intima, but was instead distributed to various extents in the media of all 10 cases in the control GSV group (Fig. 4a–c). On the other hand, the intima of the varicose GSVs showed remarkable thickening, in which abundant collagen fibrils around SMCs were present. In the media of the varicose GSVs elastic fibers were reduced, in contrast to the increase of collagen fibrils (Fig. 4d–f). While no SC was recognized in 13 of the cases in the varicose GSV group, small or moderate amounts of SC were observed in the intima and media in 5 of the cases in this group. The extent of SC in the control GSV group was significantly larger than that in the varicose GSV group (Table 3).

The control PVs contained small amounts of ECM in the subendothelial space, but showed no intimal thickening. While SC was not recognized in the intima, it was distributed in the media of 9 cases of the control PV group (Fig. 5a–c). The medial elastic fibers and stromal collagen fibrils were present in normal numbers. In contrast, all 13 cases of the LCPV group showed intimal thickening with scattered SMCs and increased collagen fibrils, in addition to a slight increase of medial collagen fibrils (Fig. 5d–f). There was a small quantity of SC in the thickened intima of only 2 cases of the LCPV group. In the media of 12 cases of the LCPV group, there were moderately to large amounts of SC. There was no significant difference between the extent of SC in the control PV and LCPV groups (Table 3). However, the proportion of cases with grade 3 SC quantity was higher in the control PV group (6/10, 60%) than in the LCPV group (3/13, 23%).

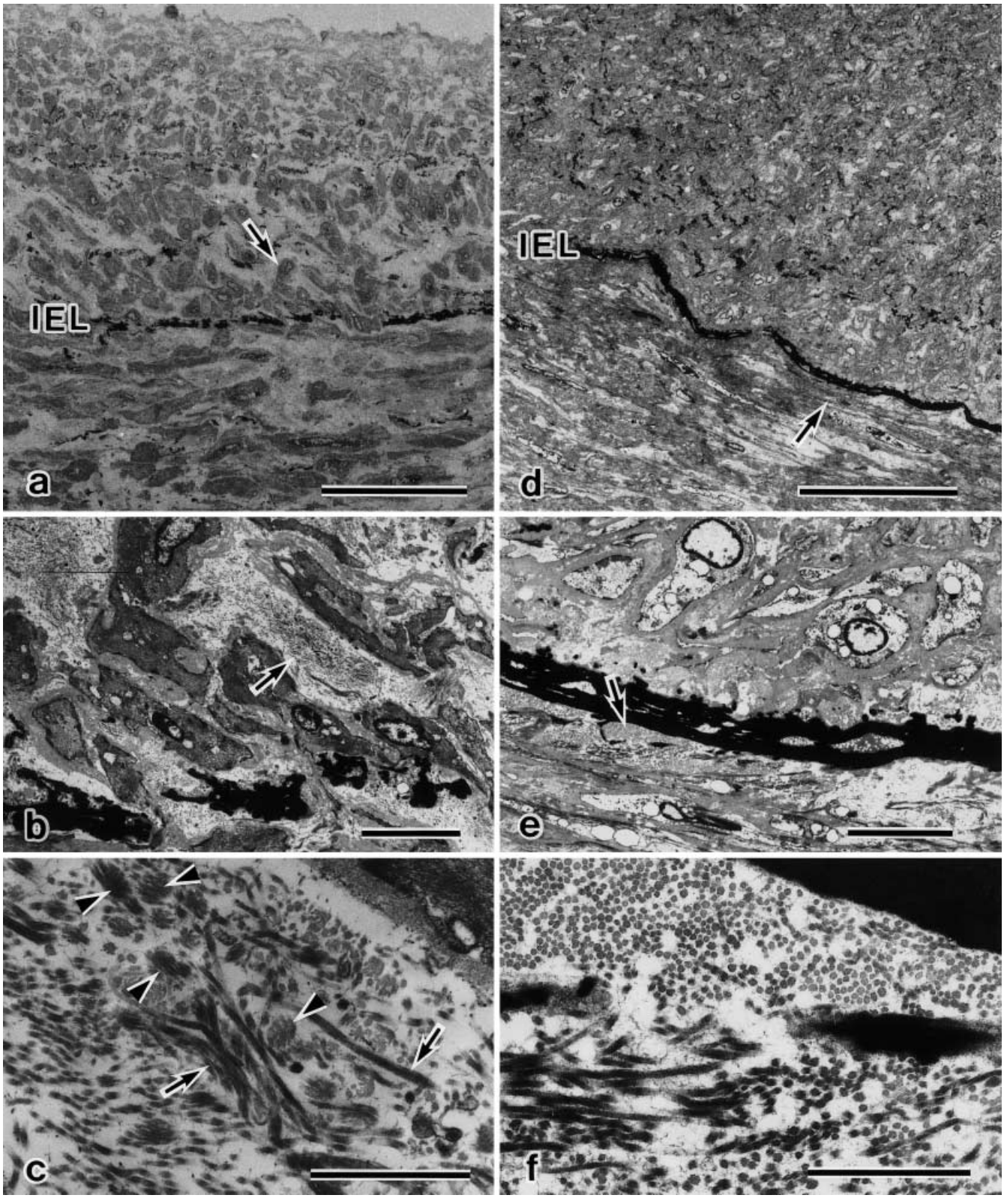

Fig. 3 Electron micrographs of LAD **a–c** under myocardial bridge [LAD MB(+)] and **d–f** LAD without myocardial bridge [LAD MB(–)]. **a** The intima of the LAD MB(+) shows mild fibrocellular thickening. Arrow portion is magnified in **b** (*IEL* internal elastic lamina). *Bar* 50 µm **b** In the intima, collagen fibrils are packed between intimal smooth muscle cells (SMCs). The portion indicated by the *arrow* is magnified in **c**. *Bar* 10 µm **c** There are abundant spiraled collagen fibrils. The features of both longitudinal (*arrows*) and cross (*arrowheads*) sections of spiraled collagen are recognized. *Bar* 1 µm **d** The intima of LAD MB(–) shows remarkable intimal thickening. The portion indicated by the *arrow* is magnified in **e**. *Bar* 50 µm **e** SMCs in the intima are of synthetic type. Interstitial collagen fibers are increased. The portion indicated by the *arrow* is magnified in **f**. *Bar* 10 µm **f** There is no spiraled collagen. Normal-sized collagen fibrils are shown. *Bar* 1 µm

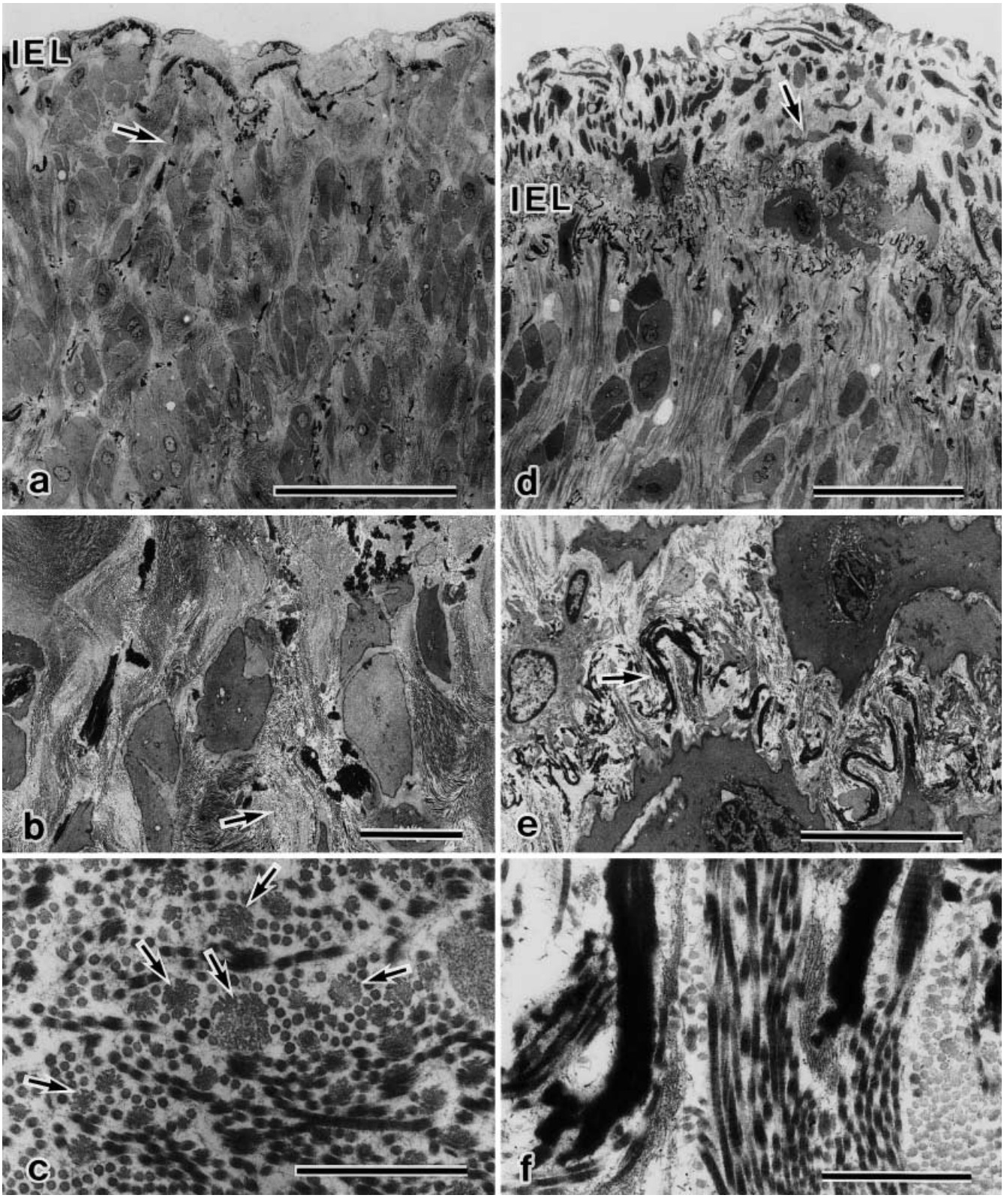

Fig. 4 Electron micrographs of **a–c** the normal great saphenous vein (*GSV*) and **d–f** a varicose GSV. **a** The normal GSV shows no intimal thickening. The portion indicated by the *arrow* is magnified in **b**. *Bar* 40 μm **b** In the media of the normal GSV, abundant collagen fibrils are distributed between SMCs. The portion indicated by the *arrow* is magnified in **c**. *Bar* 10 μm. **c** Spiraled collagen fibrils (*arrows*) exhibiting an increased thickness with a tattered rim in cross-section scattered among normal-sized collagen fibrils. *Bar* 800 nm **d** The intima of the varicose GSV is thickened, and the internal elastic lamina (*IEL*) is dispersed. The media shows an increase of interstitial components. The portion indicated by the *arrow* is magnified in **e**. *Bar* 40 μm **e** Collagen fibrils around IEL are increased. The portion indicated by the *arrow* is magnified in **f**. *Bar* 10 μm **f** Collagen fibrils are normal. There is no spiraled collagen. *Bar* 1 μm

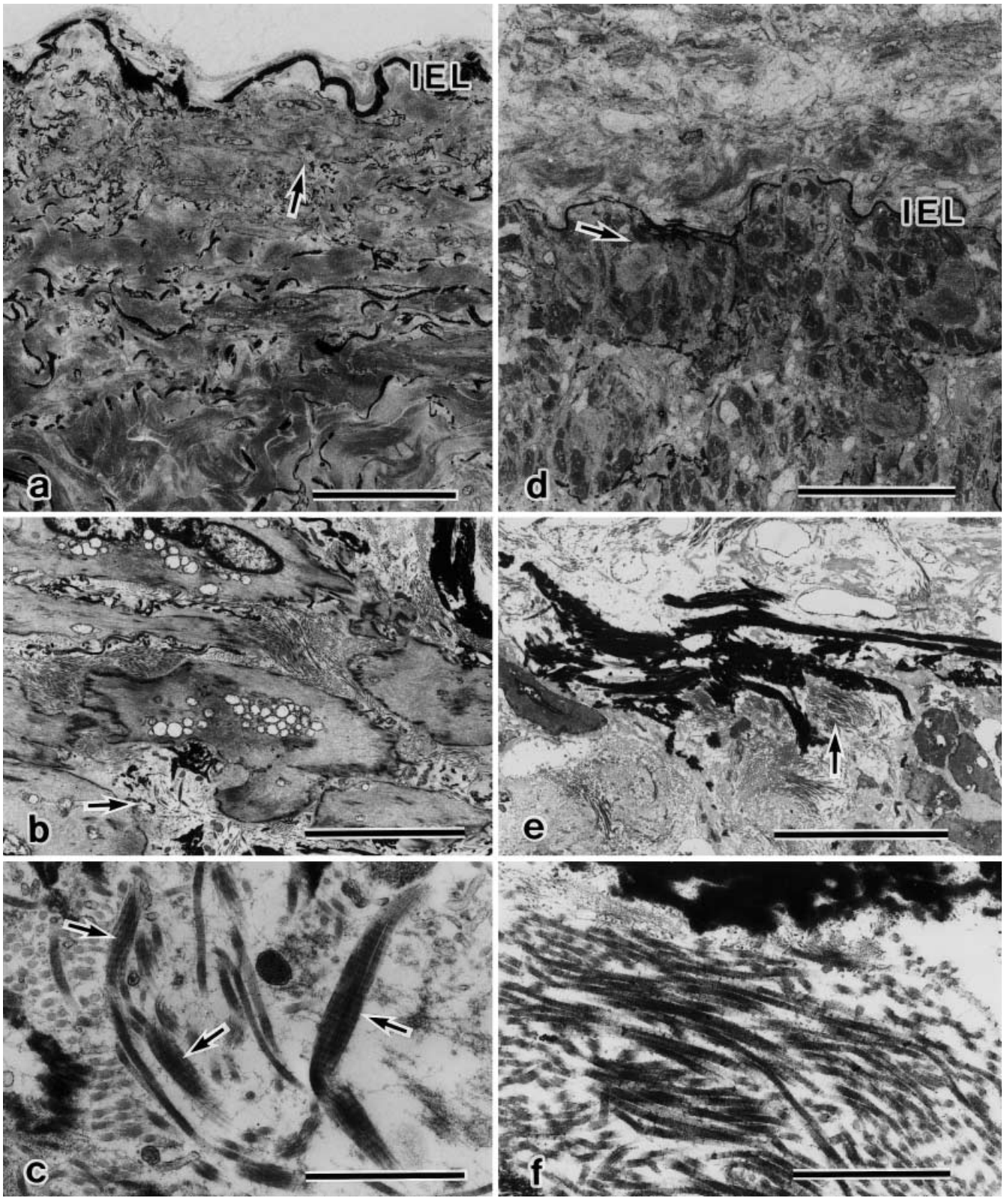

Fig. 5 Electron micrographs of **a–c** the normal portal vein (*PV*) and **d–f** PV with liver cirrhosis (*LCPV*). **a** Normal PV shows no intimal thickening (*IEL* internal elastic lamina). The portion indicated by the *arrow* is magnified in **b**. *Bar* 50 μm **b** In the media, packed collagen fibrils are recognized between SMCs. The portion indicated by the *arrow* is magnified in **c**. *Bar* 10 μm **c** Spiraled collagen fibrils (*arrows*) showing an increased thickness in longitudinal section are scattered. *Bar* 1 μm **d** The intima of LCPV shows remarkable intimal thickening, and elastic fibers in the media are reduced. The portion indicated by the *arrow* is magnified in **e**. *Bar* 50 μm **e** The media under the IEL demonstrates an increase of collagen fibrils. The portion indicated by the *arrow* is magnified in **f**. *Bar* 10 μm **f** Increased collagens are normal in size. *Bar* 1 μm

Discussion

In this study, the expression of MMPs, TIMPs and prolyl-4-hydroxylase and the extent of SC in three types of normal blood vessels were compared with those in the respective pathologic vessels. Intimal lesions were recognized in all cases in the LAD MB(–) group; however, atherosclerotic lesions were absent in the intima of the 10 cases in the LAD MB(+) group. It was evident from the endothelial morphology that the vascular wall of the LAD beneath the MB is subject to high shear stress, which has a protective effect against atherosclerotic disease [19, 20]. Although the primary cause of varix is still controversial, morphological and biochemical analyses indicate that metabolic abnormalities of SMCs in the venous wall lead to the development of varix [33, 40]. The thickened wall of the varicose GSV may be affected by increased blood pressure with complicated hemodynamic forces [33], because the development of luminal dilatation and tortuosity of the vein wall are common characteristics under varicose conditions. In addition, all cases in the LCPV group in the present study also showed intimal thickening caused simply by portal hypertension [15, 22], in contrast to the control PVs, which had intact intima. Thus, the intimal surfaces of three types of pathologic blood vessel groups, the LAD MB(–), varicose GSV and LCPV groups, were substantially influenced by different circumferential or shear stress from those of their paired control groups.

In our study, the extent of positive immunoreactivities of MMPs and TIMPs did not differ significantly between the pathologic blood vessel groups and their paired normal counterparts, except for MMP-1 expression. MMP-1 has been identified in various cells [7], and its main sources in the vascular wall are SMCs, endothelial cells and monocyte-macrophages [10]. The production of proMMP-1 by SMCs is associated with phenotypic modulation of SMCs to a synthetic state, as shown by in vivo and in vitro studies [10], and the endothelial cells overlying atherosclerotic plaques of the carotid artery express MMP-1, in contrast to its absence in normal endothelium [34]. MMP-1 expression is confined to monocyte-derived macrophages, SMCs and endothelial cells in atherosclerotic lesions of the human carotid artery, but not in nonatherosclerotic intima [28]. However, in the present study, almost all the cases of normal control vessels in the LAD MB(+), control GSV and control PV groups demonstrated constant MMP-1 expression, mainly in medial SMCs and endothelial cells, while MMP-1 expression was significantly reduced in pathologic vessel walls. It is considered that the discrepancy between our results and those of previous investigations [10, 28, 34] probably originates from differences in the materials used. The walls of normal control blood vessels used in this study are stressed by different hemodynamic conditions from those of the aorta and carotid artery used in the previous studies [10, 28, 34]: the intima of the LAD MB(+) is stressed by high shear stress, as mentioned above, and the walls of the control GSVs and PVs are influenced by lower blood pressure than that of arteries. MMP-1 synthesis by SMCs and endothelial cells is induced or stimulated by interleukin-1, platelet-derived growth factor and tumor necrosis factor alpha [7, 16, 41]. In our study, the levels of these stimulant factors for MMP-1 synthesis were not measured; however, it is considered that the differences between the hemodynamic factors stressing the intima in the pathological blood vessels and paired control blood vessels may directly and/or indirectly regulate the MMP-1 synthesis in the vascular wall.

The extents of immunoreactivity against MMP-2, MMP-9 and TIMPs did not differ significantly between pathologic blood vessels and their paired control counterparts. MMP-2 and TIMP-2 have been immunohistochemically detected in luminal endothelial cells and medial SMCs of nonatherosclerotic arteries [10]; however, a greater amount of MMP-2 is found in atherosclerotic plaques than in normal intima of the aorta [24]. In addition, a major source of MMP-2 in atherosclerotic plaques is considered to be monocyte-macrophages [24]. Our electron microscopic observations revealed that intimal lesions of the three types of pathologic blood vessels consisted almost entirely of abundant ECM and migrated SMCs. Infiltration of monocyte-macrophages in the intimal lesion was found only in 4 of the cases in the LAD MB(–) group. From these findings in the pathologic vascular walls, it is considered that the major sources of MMP-2 in the blood vessels used in this study are endothelial cells and SMCs.

The extent of immunoreactivity for prolyl-4-hydroxylase was significantly greater in the walls of the varicose GSVs and LCPVs than in their paired control vessels. Because prolyl-4-hydroxylase has been used as a marker of collagen synthesis in the vascular wall [1, 5, 39] and its activity in the vascular wall is stimulated by hypertension [26], collagen synthesis is considered to increase in the varicose GSVs and LCPVs due to increased blood pressure. In fact, electron microscopic observation also showed remarkable increases of collagen fibrils in intimal lesions of the varicose GSVs and LCPVs. On the other hand, despite the increase of collagen fibrils in intimal lesions of the LAD MB(–) group seen by electron microscopy, the extent of expression of prolyl-4-hydroxylase in the LAD MB(–) group was similar to that in the LAD MB(+) group. From these findings, in addition to the immunohistochemical findings for MMP-1, the increase of collagen fibrils in intimal lesions of the varicose GSVs and LCPVs is considered to result from the increase of collagen synthesis and decrease of collagen degradation. In contrast, it is considered that the increase in collagen fibrils in intimal lesions of LAD MB(–) depends mainly on one factor; the decrease of collagen degradation. These differences may be due in part to the differing pathologies and the different responses of arteries and veins to disturbed pressure and flow.

Our electron microscopic observations revealed that the quantity of SC was significantly greater in the LAD MB(+) and control GSV groups than in the LAD MB(–)

and varicose GSV groups. In the PVs, the proportion of cases with large quantities of SC was higher in the control PV group than in the LCPV group. These results indicate that more SC was present in the normal blood vessels in this study than in their paired pathologic vessels. The presence of SC has been reported in the pathologic vascular walls seen in conditions such as atherosclerosis [38], aneurysm in Marfan's syndrome [35], arteritis [37], Burger's disease [30], diabetic angiopathy [14] and varix [14], in addition to various normal major vessels [4, 12, 38]. Although the morphogenetic mechanism of SC is still unclear, SC is ubiquitous in the human vascular wall and it is formed preferentially in the normal vascular wall, possibly through physiological degradation of normal collagen fibrils [18]. In the present study, the decreased presence of SC in pathologic blood vessels compared with control blood vessels may have resulted from the decrease of collagen degradation due to the limited expression of MMP-1.

In the present study, the sex ratios in the pathologic vessel groups were different from those in normal control groups, because the specimens were obtained from consecutive autopsied or surgical cases. In view of the presence of estrogen receptor in the coronary arteries and the expression of estrogen and progesterone receptors in the normal and varicose GSV, it seems that the sex steroids, especially estrogens, may directly regulate the growth, differentiation and function of the cells in the vascular system [25, 31, 32]. In this study, the effect of gender may be included in the statistical analysis. However, the effects of these steroids on the collagen metabolism of endothelial and SMCs in the human vascular system are still not clear. For elucidation of the precise pathophysiological mechanism of sex-related hormones, further studies using age- and/or sex-matched samples may be necessary.

In conclusion, the intimal remodeling of blood vessels is highly dependent on the balance between collagen synthesis and degradation, and both altered blood flow leading to changes in shear stress at the endothelial surface and changes in transmural pressure and circumferential stress strongly influence collagen metabolism through modulation of MMPs and related proteins.

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