Wall Tension Is a Potent Negative Regulator of In Vivo Thrombomodulin Expression

Jason L. Sperry, Clayton B. Deming, Ce Bian, Peter L. Walinsky, David A. Kass, Frank D. Kolodgie, Renu Virmani, Antony Y. Kim, Jeffrey J. Rade

Abstract—Thrombomodulin (TM), a key component of the anticoagulant protein C pathway, is a major contributor to vascular thromboresistance. We previously found that TM protein expression is dramatically reduced in autologous vein grafts during the first two weeks after implantation, coincident to a local inflammatory response, and remains suppressed for at least 6 weeks. To determine the proximate cause of TM loss, in vivo gene expression was quantified by real-time PCR. TM gene expression in vein grafts declined >85% during the first postoperative week and remained suppressed >55% at 6 weeks, accounting for the observed changes in protein expression. The effects of vein graft inflammation were evaluated in animals rendered leukopenic with vinblastine before graft implantation. Abrogating the local inflammatory response affected neither TM protein nor gene expression. To determine how hemodynamic forces might modulate TM expression, the surgical protocol was modified to alter blood flow and pressure-induced vessel distension. TM protein and gene expression did not correlate to changes in shear stress but highly correlated to changes in wall tension, both acutely and over time. We conclude that the primary stimulus for altered TM expression in vein grafts is the exposure to arterial pressure. Furthermore, these data identify strain as a novel and important pathway for in vivo TM gene regulation. (Circ Res. 2003;92:41-47.)

Key Words: thrombomodulin • wall tension • shear stress • thrombosis • vein graft

The failure of autologous vein grafts is a major cause of morbidity and mortality in patients undergoing coronary artery bypass surgery. During the first postoperative year, 15% to 20% of grafts develop occlusive thrombosis. Thrombomodulin (TM), a 100-kDa transmembrane protein produced in abundance by endothelial cells, is critical to the maintenance of vascular thromboresistance. TM binds thrombin and alters its active site specificity to facilitate proteolytic activation of protein C, a potent inhibitor of the coagulation cascade. We recently found that TM protein expression is reduced >90% in rabbit vein grafts during the first two weeks after implantation with a gradual, but incomplete, recovery of expression by 6 weeks. Loss of TM expression reduces the capacity of grafts to activate protein C >95%, leading to an increase in local thrombin generation that may predispose vein grafts to thrombosis.

The profound early loss of TM expression in rabbit vein grafts occurs coincident to a local inflammatory response that develops in the weeks after implantation. Inflammation is an important regulator of TM expression at both the transcriptional and posttranslational levels. Inflammatory cytokines, such as tumor necrosis factor-α and interleukin-1β that are released by activated leukocytes, potently inhibit TM gene expression. In addition, activated neutrophils release proteases and reactive oxygen species that mediate shedding of the TM protein from the endothelial cell surface. The local inflammatory response observed after graft implantation might therefore contribute to early TM loss via inhibition of gene expression, protein shedding, or a combination of both.

On graft implantation, venous endothelial cells are suddenly exposed to arterial levels of blood flow and pulsatile pressure. In contrast to inflammation, there is only circumstantial evidence pointing to the importance of hemodynamic forces in regulating in vivo TM expression. Exposure of cultured endothelial cells to elevated shear stress has been reported to both increase and decrease TM gene expression, depending on the cell type. Short-term ex vivo exposure of human saphenous vein segments to arterial, but not venous, flow conditions has also been reported to cause a rapid reduction in TM protein expression and protein C activation. In addition to direct effects, hemodynamic forces might indirectly affect TM expression by stimulating vein graft inflammation. In support of this concept is the observation that rabbit vein segments reimplanted into the venous circulation do not develop the intense local inflammatory response seen in segments implanted into the arterial circulation.
We hypothesize that inflammation and mechanical hemodynamic forces, alone or in combination, modulate TM expression in vein grafts. The aim of the present study was to determine the relative importance of each and to more clearly define regulatory pathways governing in vivo TM expression. To accomplish this, we first determined the extent to which loss of TM in rabbit vein grafts is accounted for by altered gene expression versus protein shedding. We then systematically explored the effects of the vein graft inflammation and the differential effects of shear stress and pressure-induced vessel distension on both in vivo TM protein and gene expression. Our findings suggest that the primary stimulus responsible for altered TM expression in vein grafts is inhibition of gene expression mediated by pressure-induced increases in graft wall tension.

**Materials and Methods**

**Animal Model**

All animal procedures were approved by the Johns Hopkins University Animal Care and Use Committee. Interposition grafting of jugular vein segments into the carotid circulation was performed in male New Zealand White rabbits (Robinson Services, Clemmons, NC) as previously described. To evaluate the effects of inflammation, rabbits were rendered leukopenic by intravenous administration of 1 mg/kg vinblastine (Sigma-Aldrich) on preoperative day 4 and 0.25 mg/kg on preoperative day 1. Immediately before surgery, 3 mg/kg gentamicin and 250 mg cephalixin were administered intravenously for prophylaxis. Automated complete blood counts with manual differential cell counts were performed using standard techniques on citrated whole blood obtained both at the time of surgery and at harvest.

The surgical protocol was modified to alter graft blood flow and pressure-induced vessel distension. To reduce flow, the common carotid artery was ligated distal to both the interpositional vein graft and the origin of the cranial-thyroid artery as previously described. This reduced measured blood flow through the graft by approximately 80% without altering intraluminal pressure. To limit distension, grafts were externally stented using a nondistensible polyethylene 23-gauge needle cover (Vacutainer Blood Collection Set; Becton Dickinson) cut to the appropriate length. Vein segments were slips through the stent before completion of the second anastomoses. Vein grafts and control vessels were harvested at the indicated times, with a 25-gauge needle connected to a pressure transducer (Model 13-4615-50; Gould Instrument Systems) attached to a PC computer. Given that the wall thickness of ungrafted jugular veins and day 3 vein grafts is extremely thin, in situ measurements of external vessel diameters were used for shear stress and wall tension calculations. Shear stress in ungrafted jugular veins was calculated by the equation for nonpulsatile flow: \( \sigma = \frac{2Q}{\pi R^2 \mu} \) (where \( Q \) is flow in mL/sec, \( \pi \) is the radius of the vein segment in centimeters, \( \mu \) is viscosity in poise (assumed to be 0.035 for blood), and \( r \) is the radius of the vein segment in centimeters). Shear stress in vein grafts was calculated by Wormserly’s modified formula for pulsatile flow: \( \sigma = \frac{Q}{2\pi \mu R^2} \), where \( Q \) is flow in mL/sec, \( \mu \) is fluid density in g/mL (assumed to be 1.045 for blood), and \( v \) is pulsation frequency in s\(^{-1}\) (the heart rate is assumed to be 180 bpm for all animals, based on average daily measurements in conscious rabbits). Short-term changes in wall tension were estimated assuming thin-walled cylindrical vessels with constant wall thickness (Table 3) using the equation \( T = \frac{P R}{2} \), where \( P \) is intraluminal pressure in dyn/cm\(^2\) (1 mm Hg = 1333.2 dyn/cm\(^2\)) and \( R \) is vessel radius in centimeters. Changes in wall tension over time in vein grafts and carotid artery controls exposed to constant arterial pressure were estimated using the luminal radius to wall thickness ratio.

**Real-Time PCR**

Freshly harvested vein grafts and control vessels were positioned in a template, constructed by removing the bottom of a 96-well polystyrene plate, such that the luminal surface formed the bottom of the well. Because of the technical inability to reliably place ungrafted jugular vein and carotid artery segments into the template due to their small size, segments of inferior vena cava (IVC) and aorta were used for venous and arterial controls, respectively. Endothelial cell-enriched RNA was obtained by filling the wells with 0.5 mL of Trizol Reagent (Life Technologies). After a 2-minute incubation, the supernatants were removed and total RNA extracted according to the manufacturer’s instruction. Aliquots of each RNA sample were reverse-transcribed into cDNA using random hexamer primers (Superscript First-Strand Synthesis System for RT-PCR; Invitrogen). Real-time quantitative PCR was performed using a 7900HT Sequence Detector (Applied Biosystems). Triplicates of each sample were subjected to a standard PCR protocol (95°C denaturation, 60°C annealing/extension x 40 cycles) using TaqMan Universal PCR Mastermix (Applied Biosystems) containing AmpliTaq Gold DNA polymerase, primers derived from the partially-cloned rabbit TM sequence (GenBank No. AY138902; forward: 5’-GATGAATGC-GACAACGGCTAT-3’; reverse: 5’-CGGAAATGCACTCGTAGCT-3’), and a 6FAM-labeled probe to an intervening sequence (5’-CCAGGACTGTCGCGAAACCTCC-3’). The mean threshold cycle (C\(_T\)) values for each sample was compared with those of a standard curve derived from serial dilutions of cDNA made from ungrafted rabbit jugular veins and/or rabbit lung (rich in TM). For each sample, TM gene expression was normalized to endothelial cell-specific RNA content using primers and a VIC-labeled probe derived from a partially-cloned rabbit CD31 sequence generously provided by Dr Markus Hecker, University of Goettingen, Goettingen, Germany (unpublished data, 2002; forward: 5’-AACCTTACCATCAGGAA-GG-3’; reverse: 5’-CAGTTATTTCCACGTCTT-3’; VIC-labeled probe, 5’-CACACAGGCTATCCTGGACCGGT-3’).

**Western Blot Analysis**

Vein grafts and control vessels were harvested at the indicated times, cut longitudinally to expose the luminal surface, and placed in lysis buffer containing 1% Triton X-100, 100 μg/mL PMSF, and 0.1 mol/L NaCl in 20 mmol/L Tris-Hcl, pH 7.5 for 15 minutes on ice. This resulted in enrichment of endothelial cell–derived proteins. Western blot analysis was performed as previously described using primary antibodies directed against TM (No. 236; American Diag) and CD31 (JC70; Dako). TM and CD31 bands were detected by autoradiography using enhanced chemiluminescence (ECL-Plus; Amersham) and quantified by densitometric analysis using UN-SCAN-IT software (Silk Scientific).

**Morphometric Analysis**

Vein grafts and control vessels were harvested at the indicated times, perfused fixed under gentle distending pressure, and imbedded in paraffin. Three to 6 transverse sections, at least 75-μm apart, were stained with Movat’s stain. Care was taken to avoid taking sections close to the arteriovenous anastomoses. Microscopic images were captured with a Spot RT digital camera (Diagnostic Instruments).
mounted on an Olympus BX 60 microscope (Olympus America) and calibrated for distance and area. The lumen and combined neointima plus media areas were masked and measured using SigmaScan Pro 5.0 software (SPSS). Average total wall thickness was calculated by the formula:

\[
\text{Total Wall Thickness (mm)} = \frac{2 \cdot [\text{Neointima Area (mm}^2\) + \text{Media Area (mm}^2\)]}{\text{Lumen Perimeter (mm)} + \text{External Elastic Lamina Perimeter (mm)}}
\]

Vessel radius was calculated from the measured lumen area. Mean values for each graft were determined from the separate sections and used to calculate a group mean.

Statistics

All data are presented as mean±SEM. Where indicated, comparisons between two groups were by two-tailed t test. A value of \(P<0.05\) was considered statistically significant. Regression analysis was performed using Sigma plot 2001 software (SPSS).

Results

TM Gene Expression in Vein Grafts

TM protein expression falls dramatically during the first two weeks after vein graft implantation with a gradual, but incomplete, recovery of expression by 6 weeks.\(^3\) To determine if TM protein loss is accounted for by changes in gene expression, RNA was extracted from grafts harvested between 1 and 42 days after implantation and subjected to quantitative real-time PCR (Figure 1). TM gene expression, normalized to endothelial cell–specific CD31 expression, declined during the first postoperative week to 15.8±0.2% that of venous controls on day 7. As with TM protein expression, gene expression remained suppressed 6 weeks after implantation at 35% to 45% that of venous and arterial controls (\(P<0.03\) for each). Changes in TM expression were predominantly accounted for by alterations in gene expression. The exception was during the first few postoperative days where protein loss (Figures 2A and 2B) appeared to be out of proportion to changes in gene expression, suggesting that protein shedding had occurred.

Effect of Inflammation on TM Expression

A local inflammatory response develops in vein grafts during the first two weeks after implantation (online Figure 1, found in the online data supplement available at http://www.circresaha.org).\(^4\) Activated leukocytes adherent to the luminal endothelium could potentially mediate early loss of TM via shedding of the TM protein or by cytokine-mediated inhibition of TM gene expression. To determine if inflammatory cells contribute to the early loss of TM, rabbits were rendered leukopenic by administration of vinblastine before vein graft implantation (Table 1). TM protein expression was quantified by Western blot analysis in vein grafts harvested 1 and 3 days after implantation (Figures 2A and 2B). There was no difference in TM protein expression in leukopenic versus control rabbits, indicating that leukocyte-mediated shedding of the TM protein did not occur in the early postoperative period. TM gene expression in grafts harvested after 3 days was also not affected by leukopenia (Figure 2C), suggesting that cytokines elaborated by activated inflammatory cells do not affect early TM expression.

Effect of Hemodynamic Forces on TM Expression

The data suggest that inflammation is not the major regulator of TM expression in vein grafts. To determine if hemody-

![Figure 1](image1.png)

**Figure 1.** TM gene expression in vein grafts over time. Endothelial cell–enriched RNA was extracted from rabbit vein grafts harvested at the indicated times. RNA from inferior vena cava (IVC) and aorta segments were used for venous and arterial controls, respectively. TM gene expression relative to CD31 expression was determined by real-time PCR and normalized to venous controls. Values shown are the mean±SEM of \(n=4\) samples per time point.

![Figure 2](image2.png)

**Figure 2.** Effect of inflammatory cells on TM expression. Rabbits were rendered leukopenic by preoperative administration of vinblastine. A, Effect of leukopenia on TM protein expression. Representative Western blot of whole-vessel lysates of vein grafts harvested 1 and 3 days after implantation. B, Densitometric analysis of Western blots. Ratio of TM to CD31 band densities was determined for each graft and normalized to those of ungrafted jugular veins (JV). Values are the mean±SEM of \(n=3\) grafts per group. C, Effect of leukopenia on TM gene expression. TM gene expression relative to CD31 expression was determined using real-time PCR in vein grafts harvested 3 days after implantation and normalized to untreated venous controls. Values shown are the mean±SEM of \(n=4\) samples per time point.
namic forces play a more important role, several modifications were made to the standard surgical protocol to differentiate the effects of shear stress and wall tension on TM expression. Grafts were subjected to carotid artery ligation in order to reduce blood flow (Low-Flow), external stenting in order to reduce distension (Stent), or a combination of both (Stent/Low-Flow). An additional group of vein segments were implanted as arteriovenous fistula grafts to increase blood flow without significantly increasing intralumenal pressure (A-VG). Modified grafts were harvested after 3 days and normalized TM protein and gene expression compared with standard vein grafts (VG; Figure 3). External stenting resulted in a 3.5- to 5-fold increase in TM protein expression and a 2- to 2.5-fold increase in gene expression compared with unstented vein grafts that was independent of blood flow ($P<0.05$ for each comparison). TM expression in arteriovenous fistula grafts did not significantly differ from venous controls.

At the time of harvest, vessel diameter, mean pressure, and blood flow were measured in each graft, allowing for calculations of shear stress and wall tension (Table 2). Wall tension was expressed independent of wall thickness given that the latter does not change appreciably in the first 3 days after implantation (Table 3) and could not reliably be simultaneously measured in vessels used for gene expression analysis. TM gene expression did not correlate with shear stress but did exhibit a high degree of inverse correlation with wall tension (Figure 4). Because wall tension in stented vein grafts cannot be directly measured, values shown for the stented groups are considered to be upper-limit estimations based on diameter and pressure measurements. As such, the true correlation between TM expression and wall tension is likely even greater than depicted.

Vein grafts adapt to arterial pressure through a process of remodeling characterized by medial hypertrophy, neointimal hyperplasia, and dilatation. Because wall thickness increases more than lumen radius, wall tension decreases over time, eventually approaching levels found in arteries. To determine if TM expression correlates to changes in wall tension over time, morphometric analysis was performed on formalin-fixed sections of carotid arteries and vein grafts harvested between 3 and 42 days (Table 3). Because vein grafts and carotid arteries are subjected to the same intraluminal pressure, wall tension is proportional to the lumen radius to wall thickness ratio. TM protein expression was determined by quantitative Western blot analysis in a separate but identical group of vessels harvested at similar time points as previously reported. In vessels subjected to arterial pressure, TM expression exhibited a strong inverse correlation to wall tension (Figure 5). Because wall thickness continues to increase in rabbit vein grafts for up to 12 weeks after implantation, this relationship predicts that TM expression will eventually approach arterial expression levels as wall tension continues to fall. Taken together, these data suggest that the primary regulator of TM expression in vein grafts is exposure to arterial pressure, not blood flow or the effects of inflammation.

### Table 1. Absolute Granulocyte Counts

<table>
<thead>
<tr>
<th>Preoperative, cells/mL</th>
<th>Harvest, cells/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 vein grafts</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>3120±460</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>6±6</td>
</tr>
<tr>
<td>Day 3 vein grafts</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>2510±469</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>18±14</td>
</tr>
</tbody>
</table>

Values are the mean±SEM of n=3 rabbits/group.

Figure 3. Effect of hemodynamic forces on TM expression. Modifications to the surgical protocol were performed to alter blood flow and pressure-induced graft distention. TM expression was determined in vein grafts implanted for 3 days using the standard surgical protocol (VG), grafts to which the distal carotid artery was ligated to restrict blood flow (Low-Flow), grafts that were external stented to restrict vessel distension (Stent), those that were both stented and ligated (Stent/Low-Flow), and in arteriovenous fistula grafts (A-VG). A. Quantitative Western blot analysis of TM protein expression. Ratio of TM to CD31 band densities was determined for each graft and normalized to those of ungrafted jugular veins (JV). Values are the mean±SEM of n=3 grafts per group. B. Real-time PCR analysis of TM gene expression relative to CD31 and normalized to IVC controls. Values shown are the mean±SEM of n=4 samples per time point.
TABLE 2. Hemodynamic Profiles of Experimental Groups

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Diameter, mm</th>
<th>Mean Pressure, mm Hg</th>
<th>Flow, mL/min</th>
<th>Shear, dyn/cm²</th>
<th>Wall Tension, 10² dyn/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jugular vein</td>
<td>2.4±0.1</td>
<td>1.8±0.5</td>
<td>2.5±0.6</td>
<td>1.1±0.1</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>Vein graft</td>
<td>5.4±0.1</td>
<td>63.0±2.5</td>
<td>25.8±1.1</td>
<td>1.1±0.1</td>
<td>22.5±0.6</td>
</tr>
<tr>
<td>Low-flow VG</td>
<td>5.2±0.1</td>
<td>69.5±2.5</td>
<td>5.0±1.0</td>
<td>0.2±0.1</td>
<td>24.0±1.1</td>
</tr>
<tr>
<td>Stented VG</td>
<td>2.0±0.0</td>
<td>62.3±2.8</td>
<td>26.4±0.6</td>
<td>8.2±0.2</td>
<td>8.3±0.4*</td>
</tr>
<tr>
<td>Stented + low-flow VG</td>
<td>2.0±0.0</td>
<td>70.3±2.0</td>
<td>3.6±0.2</td>
<td>1.1±0.1</td>
<td>9.4±0.3*</td>
</tr>
<tr>
<td>Arteriovenous graft</td>
<td>5.6±0.2</td>
<td>5.3±1.0</td>
<td>71.3±5.0</td>
<td>2.8±0.1</td>
<td>2.0±0.5</td>
</tr>
</tbody>
</table>

Values are the mean±SEM of n=4 vessels/group. *Theoretical maximum calculated wall tension.

Discussion

The major findings of the present study are as follows: (1) beyond the immediate postoperative period, inhibition of gene expression is the predominant means by which TM expression is reduced after vein graft implantation; (2) vein graft inflammation does not significantly contribute to the early loss of TM expression; and (3) the pressure-induced increase in wall tension is a more important regulator of TM gene expression in vein grafts than is shear stress.

TM is a key component of the anticoagulant protein C pathway and therefore critically important to maintaining vascular thromboresistance. The acquired loss of TM expression and function is thought to contribute to the thrombotic manifestations of several pathological conditions, including bacterial sepsis, vasculitis, transplant rejection, preeclampsia, and radiation enteropathy.17–21 Common to all of these conditions is a prominent inflammatory component. As such, previous investigations into pathways of in vivo TM regulation have focused on the effects of inflammation. Given that a robust local inflammatory cell infiltrate develops in vein grafts during the first several weeks after implantation, it was surprising to discover that profound leukopenia had no discernible effects on either TM protein or gene expression. Although inflammation did not mediate TM protein shedding, we cannot exclude nonspecific shedding from endothelial cell injury as a possible contributor to the excessive protein loss observed in the immediate postoperative period.22

In contrast to inflammation, much less is known about how hemodynamic forces regulate in vivo TM expression. Several groups have performed in vitro studies examining the effects of shear stress on TM gene expression and found conflicting results. Exposure of cultured bovine aortic endothelial cells to shear rates between 15 to 36 dyn/cm² inhibited TM gene expression by ~80%, whereas exposure of cultured human vascular endothelial cells to 15 dyn/cm² of shear increased TM gene expression 3-fold.9,10 In neither cell type did shear rates between 1.5 to 4.0 dyn/cm² produce a change. Regardless of how one may attempt to reconcile these findings, an important caveat is that endothelial cells grown in culture may react in fundamentally different ways to changes in shear stress than do endothelial cells in an intact vessel. Through surgical manipulation of the rabbit vein graft model, we were able to expose venous endothelial cells in situ to shear stress between 0.2±0.1 and 8.2±0.2 dyn/cm². Within this range, there was no observed correlation with TM expression. Although it is possible that shear in excess of 8 dyn/cm² might modulate TM expression, these results remain relevant to saphenous vein grafts used for human coronary bypass surgery, which are subjected to calculated mean shears of 1 to 10 dyn/cm² (based on measured mean pulsatile blood flows of 20 to 70 mL/min in grafts with diameters of 3 to 5 mm²).23

The significance of the present study lies in the identification of wall tension as an important regulator of in vivo TM expression. Only one prior study has directly examined the effect of cellular distension on TM expression, although not in endothelial cells. Transcriptional profiling using DNA microarrays revealed that expression of the TM gene is downregulated by 2.6-fold in cultured human vascular smooth muscles subjected to 4% biaxial cyclic strain for 24 hours.24 It is difficult to know the physiological relevance of this finding given that vascular smooth muscle cells in situ do not ordinarily express TM, but only do so when established in culture.25

In another relevant series of experiments, Powell’s group used an ex vivo flow circuit to perfuse human saphenous vein segments under arterial and venous flow conditions for 45 to 90 minutes.11,26 Short-term exposure to pulsatile arterial flow rapidly reduced TM immunostaining by over 50% and protein C activation more than 3-fold, compared with perfusion under venous flow. External stenting only minimally prevented loss of TM immunostaining, although the size and compliance of the stent material permitted a persistent 7±2% diameter pulsatile expansion of the vein segments. Because of the brief nature of the experiments, lack of data on gene expression and possible ineffective external stenting, it is difficult to make comparisons with the present study. Nonetheless, sudden changes in either shear or vessel stretch could trigger novel regulatory pathways. In the above ex vivo studies, loss of TM immunostaining was prevented by the

TABLE 3. Vein Graft Remodeling Over Time

<table>
<thead>
<tr>
<th>Vessel (n)</th>
<th>Wall Thickness, µm</th>
<th>Radius/Wall Thickness Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jugular vein (3)</td>
<td>17.8±1.1</td>
<td>34.3±6.9</td>
</tr>
<tr>
<td>Day 3 VG (3)</td>
<td>19.3±1.4</td>
<td>42.8±7.1</td>
</tr>
<tr>
<td>Day 7 VG (3)</td>
<td>25.9±2.7</td>
<td>35.6±2.7</td>
</tr>
<tr>
<td>Day 14 VG (3)</td>
<td>90.6±29.1</td>
<td>143±2.6</td>
</tr>
<tr>
<td>Day 28 VG (10)</td>
<td>111.6±9.7</td>
<td>104±1.4</td>
</tr>
<tr>
<td>Day 42 VG (10)</td>
<td>219.6±22.6</td>
<td>92±1.6</td>
</tr>
<tr>
<td>Carotid artery (5)</td>
<td>94.9±2.9</td>
<td>3.5±0.2</td>
</tr>
</tbody>
</table>

Values are the mean±SEM.
addition of gadolinium and nifedipine to the perfusate. Given that the effect occurred too rapidly to be explained by changes in gene expression and no TM was detected in the perfusate to suggest protein shedding, this suggests the presence of a calcium-dependent pathway for protein internalization and degradation. It is conceivable that such a pathway might contribute to the excessive early loss of TM protein expression observed in rabbit vein grafts; however, more definitive studies will be needed to determine its existence and physiological relevance.

In addition to TM, wall tension may be an important regulator of other molecules involved in maintaining in vivo vascular thromboresistance. In vitro studies have shown that the eNOS, tPA, and prostacyclin synthase genes are upregulated in endothelial cells subjected to cyclic strain.\(^{27-29}\) Interestingly, porcine vein grafts implanted for 4 weeks elaborated less than half the prostacyclin of ungrafted veins.\(^{30}\) This decline in prostacyclin production was reversed by vein graft external stenting, suggesting a stretch-sensitive regulatory pathway. These data reinforce potential differences that may exist in the way endothelial cells in culture, versus those in situ, respond to hemodynamic stimuli.

The strengths of the rabbit vein graft model to investigate the effects of hemodynamic forces on TM expression revolve around its physiological relevance. Unfortunately, a major limitation of the model is the inability to parse arterial perfusion into its constitutive components. For example, it would be difficult to sufficiently modulate pulse pressure to investigate the differential effects of cyclic versus static strain on TM expression or to investigate the hierarchical effects of hemodynamic stimuli. We are attempting to overcome these limitations by modifying a sophisticated ex vivo perfusion systems to accommodate chronic perfusion of intact vein segments.\(^{31}\) Such a system is ideally suited to investigate the pathways by which wall tension regulates TM and other molecules contributing to vascular thromboresistance.

Finally, the regulation of TM expression by changes in wall tension may have implications beyond that of vein graft disease. Pulmonary hypertension, atrial fibrillation, and venous insufficiency are examples of conditions characterized by elevated intraluminal pressure and in situ thrombosis. It is intriguing to speculate that impaired TM expression might contribute to the prothrombotic tendencies associated with these conditions. Elevated plasma levels of soluble TM, thought to represent endothelial cell injury, are elevated in patients with both pulmonary hypertension and atrial fibrillation.\(^{32-34}\) However, in the subset of patients with very high intraluminal pressures from primary pulmonary hypertension and mitral stenosis, soluble TM levels are significantly lower than even those of healthy controls.\(^{32,35,36}\) One wonders if this could reflect a decrease in endothelial cell expression of TM. Further studies are clearly needed to define the relationship between tissue TM expression, circulating soluble TM, and procoagulability in patients with these conditions.

In summary, we demonstrate that pressure-induced increase in vascular wall tension is a potent negative regulator of TM gene expression. This is the primary mechanism by which TM expression is altered in rabbit vein grafts. These findings may help to explain the high rate of early vein

Figure 4. Relationship between short-term changes in hemodynamic forces and TM expression in vein grafts. Normalized TM gene expression was measured by real-time PCR 3 days after implantation in standard vein grafts, vein grafts subjected to restrictions in blood flow and vessel distension, arteriovenous fistula grafts, and ungrafted jugular veins. Shear stress and wall tension were calculated from pressure, flow, and size measurements obtained in the same vessels at the time of harvest. TM gene expression in individual grafts was plotted against shear stress (A) and wall tension (B).

Figure 5. Relationship between changes in wall tension and TM expression in vein grafts over time. Normalized levels of TM protein expression was determined by quantitative Western blot analysis in carotid arteries (open circle) and vein grafts (filled circles) harvested between 3 and 42 days after implantation. Wall tension was estimated by the lumen radius/wall thickness ratio determined by morphometric analysis of formalin-fixed sections obtained from vessels harvested at similar time points. Because protein expression and morphometry could not be performed on the same vessels, values are plotted as group mean±SEM.
graft failure in patients undergoing coronary and peripheral arterial bypass procedures. Because TM expression predictably recovers as vein grafts arterialisate, a rationale exists to develop short-term pharmacological or gene transfer–based therapies to prevent graft thrombosis during this vulnerable period.

Acknowledgments

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Figure Legends

Figure 1. Local inflammatory response in rabbit vein grafts. A. Hematoxylin and eosin stained cross section of a formalin-fixed vein graft harvested 3 days after implantation. Numerous inflammatory cells can be seen adherent to the lumenal endothelial cells and infiltrating the vessel wall. B. Scanning electron micrograph of the lumenal surface of a rabbit vein graft harvested 7 days after implantation. Inflammatory cells, platelets and fibrin can be seen carpeting an injured, but intact, endothelial cell layer.