Fibrinogen, a plasma 340-kDa glycoprotein, is converted to fibrin on limited proteolysis by thrombin. The protein is heterogeneous because of variations in partial proteolysis, phosphorylation or sulfation of amino acids, genetic polymorphisms, and alternative splicing. Fibrinogen consists of 2Aα-, 2Bβ-, and 2γ-chains, linked in a dimeric structure by 29 disulfide bonds. The Bβ and γC termini compose the D-region, whereas the E-region contains the N termini of all 6 chains. The D-regions are connected to the E-region by 2 α-helical coiled segments (Figure 1). The αα, αβ, and γC termini are globular and located close to the E-region in fibrinogen.

Fibrin formation is initiated by thrombin-mediated release of FpA and FpB from the Aα and Bβ N termini, respectively. In solution, cleavage of FpA occurs first, inducing polymerization into protofibrils of half-staggered, overlapping fibrin units (Figure 1). FpB is cleaved at a slower rate than FpA by thrombin. Fibrinopeptide release undergoes different kinetics when fibrinogen is bound to a surface. Riedel et al recently showed that FpB release is increased, particularly from fibrinogen with “end-on” as opposed to “side-on” surface attachment. Surface-related fibrin deposition may play a role in stent or cardiopulmonary bypass thrombosis, on cells and subendothelial structures. FpB release is associated with lateral aggregation of protofibrils, which is caused by interacting α C-terminal domains (Figure 1). Lateral aggregation contributes to fiber thickness and tensile strength of fibrin. The overall mechanical properties of fibrin are determined by structural features at the levels of the molecule, individual fibers, and the branched fiber network. A key inhibitor of tPA, plasminogen activator inhibitor-1 (PAI-1), is released from platelets and the endothelium on activated factor (F)XIII improves the elastic properties and resistance to fibrinolysis. γ-Chain cross-links occur between lysine 406 on one chain and either glutamine 398 or 399 on another. α-Chain cross-linking results in oligomer and polymer formation. FXIIIa also cross-links α2-antiplasmin, thrombin-activatable fibrinolysis inhibitor (TAFI), and plasminogen activator inhibitor-2 to fibrin, contributing to its resistance to fibrinolysis. The importance of cross-linking by FXIIIa is highlighted by the severe bleeding associated with its deficiency.

Fibrinolysis is mediated by interaction of tissue plasminogen activator (tPA) and plasminogen. Fibrin greatly accelerates the conversion of plasminogen to plasmin by tPA. Plasmin cleaves Lys-X and Arg-X bonds in fibrin, breaking down the fiber structure.

Plasminogen and tPA bind to lysine residues exposed by fibrinolysis, further accelerating the conversion of plasminogen to plasmin. Plasmin that is bound to fibrin is relatively protected from inhibition by circulating α2-antiplasmin. However, α2-antiplasmin also binds to fibrin and helps to protect the clot against fibrinolysis.

A key inhibitor of tPA, plasminogen activator inhibitor-1 (PAI-1), is released from platelets and the endothelium on
stimulation with cytokines or thrombin. Urokinase-type plasminogen activator primarily participates in cell-mediated plasmin generation.28 Urokinase-type plasminogen activator is expressed by kidney and tumor cells and participates in angiogenesis by binding to its endothelial receptor urokinase-type plasminogen activator receptor. Urokinase-type plasminogen activator lyses the clot, allowing tubular in-growth of new endothelium. TAFI downregulates plasmin generation by removing C-terminal lysine residues on fibrin, resulting in increased stability of thrombi.29

Fibrin structure itself directly affects fibrinolysis rates,30 and mechanisms that control this have recently been reviewed.31 Longstaff et al32 recently showed that accessibility of the clot to fibrinolytic proteins and alterations in binding of tPA and plasminogen were both regulated by fibrin structure. Fibrinolysis proceeds rapidly in platelet-poor areas of the clot, whereas platelet-rich areas remain relatively unlysed.33 Fibrin networks composed of the thin, highly branched fibers are less permeable, more rigid, and less susceptible to lysis. Clots composed of thick fibers have larger pores, leading to higher permeability and accelerated fibrinolysis.30–33 Fibrinogen concentrations explain up to 18% of the variation in clot permeability.34

Modulators of Fibrin Properties

A number of genetic and environmental factors correlate with fibrin structure and its association with thrombotic disease. These are discussed below and summarized in Figure 2.

Genetic Factors

Genetic factors contribute moderately to variance in fibrin structure.34,35 Quantitative trait loci for fibrin structure are located in chromosomes 5, 6, 9, 16, and 17.36 Odds ratios of more than 1000:1, expressed logarithmically as an LOD score of >3, are considered significant genetic linkage in humans. Six regions with LOD >3 have been identified.36 For most fibrin characteristics, heritability ranges from 10% to 40%.34,37 Each fibrinogen chain is encoded by a separate gene, all 3 of which are located in the same region on chromosome 4 (4q28.1, 4q28.2, and 4q28.3 for FGG, FGA, and FGB, respectively).38 Fibrinogen synthesis involves assembly of the hexamer in the endoplasmic reticulum, with α-chain incorporation as rate-limiting step.39 Dysfibrinogenemias resulting from mutations in the fibrinogen genes are linked with arterial or venous thrombosis in 25% of cases. An excellent review on inherited fibrinogen abnormalities has been published recently.40

Fibrin structure may also be influenced by common genetic variation. A common β-chain polymorphism, Lys448Arg, has been shown to affect clot structure in plasma.41 Recombinant Lys448 and Arg448 fibrinogens also showed differences in fibrin structure, both in purified systems and in plasma. The recombinant variants significantly affected lysis times when reconstituted in plasma.42 There is further evidence linking clot structure with an α-chain Thr312Ala polymorphism. The Ala312 allele has been associated with...
thicker fibrin fibers, increased α-chain cross-linking by FXIIIa,\(^4\) and higher mortality in patients with atrial fibrillation complicated by ischemic stroke, by predisposing to arterial embolization.\(^4\)

A fibrinogen γ-chain splice variant, γ', leads to the substitution of 4 C-terminal residues containing a platelet binding site with 20 new residues that contain both thrombin and FXIII B-subunit binding sites.\(^2\) Clots produced with γ-fibrinogen have thinner fibers, more branch points, and increased resistance to lysis.\(^2\) Based on SEM identification of capped fiber ends, charge-charge repulsion by the negatively charged γ' chain were suggested to play a role in impaired polymerization,\(^4\) a hypothesis that deserves further investigation. Taken together, these studies show that common genetic variations in all fibrinogen polypeptide chains can influence fibrin structure and function, which ultimately may translate into altered risk for thrombosis.

FXIII polymorphisms have also been associated with alterations in fibrin structure. A G>T transition in codon 34, with subsequent replacement of valine with leucine (FXIII Val34Leu), significantly alters fibrin structure. Thrombin demonstrates higher catalytic efficiency when activating FXIII Leu34 compared with Val34.\(^3\) Early activation of FXIII results in the formation of clots with smaller pores and thinner fibers.\(^4\) There is evidence that the Leu34 allele protects against myocardial infarction (MI) and venous thrombosis.\(^5\) Increased FXIII activation in Leu34 carriers may result in ineffective cross-linking.\(^4\) The apparent discrepancy between increased FXIII activation and protection against MI may also be due to interactions between Val34Leu and fibrinogen concentrations.\(^6\) At high fibrinogen concentrations, FXIII Leu34 leads to the formation of more permeable clots that are more susceptible to lysis, whereas at a low fibrinogen concentration, the effects were reversed, suggesting that protection against thrombosis by FXIII 34Leu occurs only at elevated fibrinogen levels.

### Pathophysiological Modulators

**Thrombin**

(Pro)thrombin concentration has a major impact on fibrin structure. Wolberg et al showed that fibrin fiber diameter decreases with increasing prothrombin levels.\(^5\) In both purified fibrinogen and plasma-based systems, clots produced with high thrombin concentrations are characterized by thin fibers that form a network with small pores.\(^4\) There potentially are many factors that influence both thrombin generation and fibrin clot structure. Examples of the latter include anticoagulant drugs that have been shown to influence fibrin structure through reduced thrombin generation. Of note, some other modifiers of fibrin structure/function, such as statins, might also at least in part alter fibrin characteristics through reduced thrombin activity (see below).

Reduced thrombin generation in hemophilia B has been associated with the formation of loosely packed fibrin susceptible to lysis.\(^5\) In addition, reduced TAFI activation in hemophilia leads to increased susceptibility to fibrinolysis.\(^5\) Recombinant activated FVII increases thrombin generation rates, normalizing fibrin structure and increasing clot stability.\(^5\)

The Factor V Leiden and G20210A prothrombin mutations are the most common genetic thrombophilic factors in whites that increase thrombin generation.\(^5\) To our knowledge, there are no studies on fibrin structure in carriers of these mutations. One interesting report showed that whereas carrier status of Factor V Leiden increases venous thromboembolism (VTE) risk 3.5-fold, when combined with hypofibrinolysis, the risk is increased 8.1-fold.\(^5\) In contrast, prolonged lysis time together with prothrombin 20210A did not synergistically heighten risk.\(^5\) Individuals with the prothrombin G20210A allele showed normal fibrin elastic moduli.\(^5\)

Thrombin generation is a dynamic, localized process, and the formation of fibrin is determined by cellular procoagulant activity, which leads to spatial heterogeneity in clot structure associated with the distance of fibrin from the cell surface. A denser fibrin network is formed within 10 μm of the cell, as shown in experiments on human fibroblasts incubated with the prothrombinase complex and fibrinogen or plasma.\(^6\) Stimulation of endothelial cells with cytokines causes the formation of compact fibrin networks resistant to lysis.\(^6\) The molecular mechanisms that regulate clot structure close to the endothelium are unknown but could involve local tissue factor activity, changes in thrombomodulin concentration, or the endothelial fibrinogen receptor αvβ3.
Blood Flow
Fibrin fibers are aligned in the direction of flow, which has important implications for clot elastic properties and response to fibrinolysis. Fibrin fibers are more resistant to stretch than flexion, and hence fiber alignment in the direction of flow will increase clot stiffness in that direction. One study found no effect of flow on fiber diameter, whereas another reported formation of thicker fibers in the direction of flow, with thinner fibers interconnecting these larger fibers perpendicularly. Changes in fiber diameter influence plasmin generation and the resistance to fibrinolysis.

Oxidative Stress
Fibrinogen is particularly susceptible to oxidation, particularly at sites of platelet aggregation. Investigators reported that exposure of fibrinogen to Fe(III) ascorbate promotes fibrin formation, enhances platelet aggregation, and supports less efficient plasminogen activation by tPA. Studies on air pollution have shown that ultrafine particulate matter can modulate fibrin structure in an oxidation-dependent manner. Addition of antioxidants reversed this effect.

Nitration of 2 β-chain tyrosines increases fibrin formation and stiffness, impairs clot lysis, and alters fibrin structure. Data on the association between oxidative stress markers and fibrin clot properties in vivo are scarce. F2-isoprostanes, produced on nonenzymatic arachidonic acid peroxidation and a stable marker of oxidative stress, have been shown to associate with reduced clot permeability and fibrinolysis in cardiovascular patients. Taken together, these studies suggest that oxidative stress may promote prothrombotic alterations in fibrin formation and architecture.

However, clinical trials failed to show benefits from antioxidant therapies in diseases believed to be associated with oxidative stress such as atherosclerotic vascular disease. It has been postulated that the antioxidant therapy did not last long enough to reveal the beneficial effects in cardiovascular patients. Moreover, in vitro evidence suggests that fibrinogen oxidation may both impair and enhance the formation of stable fibrin clots; thus, in the presence of additional modifiers of fibrin, the net effect could be different in subjects at various cardiovascular risk. Additional studies are needed to elucidate the in vivo effects of oxidative stress on fibrin structure and function.

Platelet Activation
Proteins released from platelets alter clot properties, particularly at sites of platelet aggregation. Increased amounts of platelet factor 4 are associated with the formation of a compact clot structure. Polyphosphate, a negatively charged polymer of inorganic phosphate secreted from dense granules, also modifies the fibrin network and its plasmin-mediated degradation. The effects of polyphosphate on clot structure are calcium dependent and independent from FXIII activation. Polyphosphates lead to the formation of tight fiber aggregates interspersed with large pores. Fibrinolysis is impaired because of reduced binding of plasminogen and tPA to partially lysed fibrin. Pyrophosphate, also released from activated platelets, blocks polyphosphate-induced enhancement of fibrin polymerization. The effect of polyphosphate depends on polymer length and the highest fibrin turbidity is induced by polyphosphate of >250-mers, although 65-mers, the size of polyphosphate released by platelets, also show significant effects. Platelets also release PAI-1 that contributes to impaired fibrin degradation and the role of PAI-1 in clot lysis increases with the number of platelets.

Lipoprotein(a)
Lipoprotein(a) contains apolipoprotein(a), whose Kringle domains are homologous with plasminogen Kringle IV and V. Elevated lipoprotein(a) levels correlate with decreased fibrin permeability, thinner fibers, and reduced susceptibility to fibrinolysis. The relationship between lipoprotein(a) and clot properties depends on apolipoprotein(a) isoforms, whereby small isoforms are responsible for abnormal fibrin characteristics. Molecular mechanisms underlying apolipoprotein(a)-related changes in clot properties remain unclear. The fibrinogen αC-regions contain apolipoprotein(a)-binding sites. Additional studies are required to investigate how these binding sites may play a role in fibrin structure and fibrinolysis.

Modulation Related to Other Mechanisms of Disease
Diabetes Mellitus
Abnormal fibrin clot properties have consistently been associated with diabetes. Increased fibrinogen levels observed in type 2 and type 1 diabetes correlate with the degree of hyperglycemia. Clots formed from patients with diabetes using purified fibrinogen or plasma are less porous than controls. Altered fibrin structure in diabetes is attributed to fibrinogen glycation, which interferes with fibrin polymerization, cross-linking by FXIII, tPA and plasminogen binding, and plasminogen to plasmin conversion. Fibrinogen glycation occurs in vivo and correlates with hyperglycemia. Fibrinogen purified from patients with diabetes produces clots that are denser and resistant to fibrinolysis. These studies point to pathophysiological mechanisms whereby fibrinogen glycation produces abnormal clot structures that contribute to thrombosis risk (Figure 3). Treatment with insulin makes fibrin more permeable through changes in fibrinogen levels.

Hyperhomocysteinemia
Homocysteine (Hcy), produced through methionine metabolism, is associated with an increased risk for coronary artery disease (CAD) and thrombosis. In rabbits, hyperhomocysteinemia is associated with the formation of fibrin with thinner and more tightly packed fibers and increased resistance to fibrinolysis. Hcy addition in vitro results in the formation of plasma clots with shorter fibers and a more compact structure. The e-amino group of fibrinogen lysines can be modified by a highly reactive thioester, Hcy thiolactone, present in small amounts in plasma. Ten lysines in the D- and αC-regions can be homocysteinylated. Homocystei-
lysinability.88 Hcy lowering trials, however, failed to reduce Hcy resulted in increased clot permeability and improved clot effects of Hcy-lowering could be attenuated by other pro-

Figure 3. Mechanisms of fibrinogen glycation, fibrin structure, and risk of thrombosis. Diabetes and insulin resistance are associated with hyperglycemia. Hyperglycemia over a prolonged period of time will lead to nonenzymatic glycation of plasma proteins, including fibrinogen. Glycated fibrinogen leads to denser fibrin clots that are stiffer and more resistant to fibrinolysis and that will increase the thrombotic burden.

Smoking-Related Diseases
Cigarette smoking increases thrombotic risk via multiple mechanisms, including a marked increase in fibrinogen levels. It has been reported that following acute exposure to cigarette smoke, fibrin clots are denser and composed of thinner fibers compared with nonsmoking and presmoking samples.91 Thromboelastography performed in whole blood before and after smoking 2 cigarettes showed lower lysis efficiency.92

In apparently healthy men who reported cigarette smoking for 5 years or more, current smoking is associated with 22% lower clot permeability and 35% longer clot lysis compared with never smokers.93 These smoking-related fibrin abnor-

Drug-Related Modulation
Acetylsalicylic Acid (Aspirin)
Aspirin increases clot permeability and fiber mass-length ratio by up to 65% (Table 1).94 Of note, a dose of 320 mg/day exerts a weaker effect on fibrin properties than a lower dose of 75 mg/day.94,95 The mechanism behind this nonlinearity is unknown. Seven days after aspirin withdrawal, clot permeability returns to baseline.96 Aspirin-related increases in clot pore size in stable CAD have also been associated with enhanced clot lysability.97

Table 1. Interventions and Factors That Have Been Suggested as Potential Modifiers Favorably Altering Fibrin Clot Properties

<table>
<thead>
<tr>
<th>Modulatory Mechanisms</th>
<th>Interventions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease in thrombin generation</td>
<td>Treatment with anticoagulants,110–112 statin use104,107</td>
</tr>
<tr>
<td>Reduction in oxidative stress</td>
<td>Cessation of smoking95–97</td>
</tr>
<tr>
<td>Improved glycemic control</td>
<td>Treatment with insulin83 and metformin108</td>
</tr>
<tr>
<td>Decrease in total homocysteine</td>
<td>Administration of folate acid84</td>
</tr>
<tr>
<td>Decrease in C-reactive protein</td>
<td>Statin use104,105</td>
</tr>
<tr>
<td>Acetylation of proteins</td>
<td>Aspirin94–99</td>
</tr>
<tr>
<td>Other or unknown</td>
<td>Fibrates, angiotensin-converting enzyme inhibitors104</td>
</tr>
</tbody>
</table>

malities appear to be determined largely by elevated fibrino-
gen and enhanced oxidative stress.93
Of note, clot properties correlated with decreased thrombin generation, most likely induced by downregulation of tissue factor. In subjects with low-density lipoprotein cholesterol below 3.4 mmol/L, simvastatin was also shown to increase clot permeability associated with faster lysis. This correlated with a reduction in C-reactive protein (CRP) (see below). Increased fibrin permeability following statin administration can be observed even in diabetic patients with dyslipidemia. Interestingly, fibrates such as gemfibrozil have no effects on fibrin permeability except for microwaved fenofibrate, which exerts additional antithrombotic and anti-inflammatory actions. It is unclear whether fibrin-modulating effects contribute to cardiovascular benefits of statins, and additional studies are required to address this.

**Angiotensin-Converting Enzyme Inhibitors**

Data on the effect of angiotensin-converting enzyme inhibitors on fibrin are sparse. Quinapril at 10 mg/day for 1 month increased clot permeability independently of antihypertensive effect. Depressed thrombin formation after treatment with angiotensin-converting enzyme inhibitors in CAD patients associated with improved clot permeability. It might be speculated that antithrombotic effects could contribute to clinical efficacy of angiotensin-converting enzyme inhibitors; however, future studies will be required to investigate this further.

**Glucose-Lowering Agents**

Glucose lowering agents might indirectly affect clot structure by decreasing fibrinogen levels or extent of fibrinogen glycation, however data concerning this are inconsistent. Metformin affects the fibrin structure by different mechanisms. Metformin interferes with fibrin polymerization and reduces FXIII-mediated cross-linking leading to increased lysability. Increased clot permeability and decreased lysis times were also observed in patients with advanced CAD aged 60 years or older. Fibrin is a consistent component of atherosclerotic plaques, and its presence may promote plaque growth.

Collet et al reported that clots from 33 young survivors of MI who survived such thrombotic event. Patients with stent malapposition or underexpansion were associated with less permeable and lysable clots in plasma drawn within the first 12 hours from the onset of chest pain. Moreover, clots from patients with acute MI contained thicker fibers and began polymerization faster than those of stable angina. Fibrin fibers in the lumen. Interestingly, these findings indicate that apart from other factors associated with stent thrombosis (including the procedure itself, patient and lesion characteristics, stent design, and premature cessation of antiplatelet drugs), fibrin-related factors might contribute not only to late thrombosis but also acute and subacute thrombosis, in particular when stent malapposition or underexpansion are excluded. Similarly, abnormal fibrin structure has been observed in patients with a history of the no-reflow phenomenon, defined as the absence of a complete myocardial perfusion despite successful opening of the infarct-related artery.
Table 2. Studies on Associations Between Vascular Disorders and Fibrin Properties

<table>
<thead>
<tr>
<th>Disease</th>
<th>No. of Subjects</th>
<th>Study Design</th>
<th>Measurements</th>
<th>Clot Phenotype</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute MI</td>
<td>40</td>
<td>Case-control</td>
<td>Permeation, turbidity, lysis assays, microscopy</td>
<td>↓ Kₚ ↑ lysis time ↑ fiber thickness ↓ lag phase</td>
<td>70</td>
</tr>
<tr>
<td>Previous MI</td>
<td>38</td>
<td>Case-control</td>
<td>Permeation</td>
<td>↓ Kₚ</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>Case-control</td>
<td>Rigidity, lysis assays, microscopy</td>
<td>↓ rigidity</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lysis assay</td>
<td>↑ lysis time</td>
<td></td>
</tr>
<tr>
<td></td>
<td>198</td>
<td>Case-control</td>
<td>Lysis assay</td>
<td>↑ lysis time</td>
<td>129</td>
</tr>
<tr>
<td></td>
<td>555</td>
<td>Case-control</td>
<td></td>
<td>↑ lysis time</td>
<td>118</td>
</tr>
<tr>
<td>Acute stroke</td>
<td>45</td>
<td>Case-control</td>
<td>Permeation, turbidity, lysis assays, compaction, microscopy</td>
<td>↓ Kₚ ↓ compaction</td>
<td>127</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑ lysis time</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↓ lag phase</td>
<td>126</td>
</tr>
<tr>
<td>Previous stroke</td>
<td>147</td>
<td>Case-control</td>
<td>Permeation, turbidity, lysis assays, microscopy</td>
<td>↓ Kₚ ↑ lysis time ↑ fiber thickness ↓ lag phase</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑ lysis time</td>
<td></td>
</tr>
<tr>
<td>In-stent thrombosis</td>
<td>103</td>
<td>Case-control</td>
<td>Lysis assay</td>
<td>↓ Kₚ</td>
<td>129</td>
</tr>
<tr>
<td>Advanced CAD</td>
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<td>Case-control</td>
<td>Permeation, turbidity, lysis assays, compaction</td>
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<td>121</td>
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<td></td>
<td>↓ lag phase</td>
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<td>Case-control</td>
<td>Permeation, lysis assay, microscopy</td>
<td>↓ Kₚ</td>
<td>115</td>
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<td></td>
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<td>↑ lysis time</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>↑ fiber thickness</td>
<td>113</td>
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<tr>
<td>Peripheral arterial disease</td>
<td>106</td>
<td>Cohort</td>
<td>Permeation, turbidity, lysis assays</td>
<td>↓ Kₚ ↑ lysis time ↓ lag phase</td>
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<td>↓ Kₚ ↓ fiber thickness</td>
<td>128</td>
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<td></td>
<td>↑ lysis time</td>
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<td>↑ lysis time</td>
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<td>↑ lysis time</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>↑ lysis time</td>
<td></td>
</tr>
<tr>
<td>Venous thromboembolism*</td>
<td>100</td>
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<td>Permeation, turbidity, lysis assays, compaction</td>
<td>↓ Kₚ ↓ compaction</td>
<td>135</td>
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<tr>
<td></td>
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<td>↑ lysis time</td>
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<td>↑ fiber thickness</td>
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<td>↑ lysis time</td>
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<td>↑ lysis time</td>
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<td></td>
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<td>↑ lysis time</td>
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<td>Lysis assay</td>
<td>↑ lysis time</td>
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<td>2090</td>
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<td>↑ lysis time</td>
<td>58</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>20 (type 1)</td>
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<td>Permeation</td>
<td>↓ Kₚ</td>
<td>79</td>
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<tr>
<td></td>
<td>150 (type 2)</td>
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<td>80, 81</td>
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<td></td>
<td></td>
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<td>↑ lysis time</td>
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<td>↓ Kₚ ↓ compaction</td>
<td>136</td>
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<td></td>
<td></td>
<td>↑ fiber thickness</td>
<td></td>
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<td></td>
<td>↓ Kₚ ↓ compaction</td>
<td></td>
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<tr>
<td></td>
<td>33</td>
<td>Case-control</td>
<td>Permeation, turbidity, compaction, lysis assays, microscopy</td>
<td>↓ lag phase</td>
<td>137</td>
</tr>
</tbody>
</table>

(Continued)
Ischemic Stroke

Plasma obtained following cerebrovascular ischemic events formed 20% denser clots compared with controls. Fibrin properties showed an association with stroke severity but not with poststroke mortality during a 7-year follow-up. Ischemic stroke of unknown origin, representing one quarter of all cases, might be particularly associated with abnormal fibrin features. Patients with cryptogenic stroke showed dense clots resistant to lysis. Ischemic stroke in the acute phase is associated with abnormal fibrin properties, which are similar to those encountered in acute MI subjects, indicating that reduced clot permeability and lysis represent common features in patients with cardiovascular disease complicated by ischemic events.

Patients with acute stroke and concomitant CAD showed prolonged clot lysis compared with those without a history of CAD. Fibrin clot compaction correlated with neurological deficit both on admission and at discharge of patients admitted for acute ischemic stroke. Lower clot permeability and reduced fibrinolysis observed in the acute phase of ischemic stroke do not change after 60 days from the event, suggesting that hypofibrinolysis is a persistent characteristic of ischemic stroke. Overall, ischemic stroke is linked with fibrin structure alterations that underlie common mechanisms leading to cerebrovascular and coronary thromboembolic episodes. Given the fact that ischemic stroke is a highly heterogeneous pathology, it is unclear whether all types of ischemic strokes share similar fibrin characteristics.

Peripheral Arterial Disease

Peripheral arterial disease (PAD) has a prevalence between 3% and 10% in the general population and is associated with a 6-fold increase in cardiovascular mortality. Plasma obtained from patients with PAD formed fibrin clots with reduced permeability and susceptibility to lysis. During follow-up, clot phenotype was associated with an increased risk for thromboembolic events and the progression of PAD. Bhainsin et al reported on 34 relatively young patients with mild to moderate PAD in whom plasma fibrin clots were poorly permeable, rigid, and resistant to lysis, with increased fiber thickness.

Hypofibrinolysis was associated with a 2.3-fold higher odds ratio of PAD. First-degree relatives of PAD patients showed similar clot characteristics, providing evidence for genetic regulation of fibrin characteristics in PAD. PAD therefore appears associated with unfavorable clot characteristics, reflected to some extent by the overall atherosclerotic burden.

VTE

Thrombophilia screening fails to identify predisposing factors in 30% to 50% of patients with idiopathic VTE, including deep-vein thrombosis and pulmonary embolism. Curnow et al showed that hypercoagulable patients with arterial thrombosis or VTE, pregnancy complications, or autoimmune diseases have increased fibrin generation and reduced fibrinolysis. Several studies documented reduced efficiency of clot lysis in VTE patients. Hypofibrinolysis has been shown in subjects following the first deep-vein thrombosis episode. A 2-fold increased deep-vein thrombosis risk has been found in subjects with clot lysis times above the 90th percentile. Up to 77% of clot lysis time variation in venous thrombosis patients can be attributed to PAI-1, TAFI, prothrombin, and α2-antiplasmin levels, with minimal contribution of fibrinogen levels. Three established risk factors for VTE, namely oral contraceptives, immobilization, and FV Leiden, markedly increase the risk associated with longest clot lysis time. After excluding known thrombophilia, cancer, trauma, surgery, pregnancy, and other established risk factors, VTE patients and their first-order asymptomatic relatives are characterized by lower clot permeability, lower compaction, higher maximum clot absorbancy, and prolonged clot lysis time than controls, with more pronounced abnormalities in patients versus relatives. Interestingly, fibrin clots obtained for pulmonary embolism patients were more permeable, were less compact, and lysed more efficiently compared with those of deep-vein thrombosis patients. These findings support the concept of similar pathophysiology involving alterations of fibrin structure in both arterial and venous thrombosis. It is unclear whether VTE patients with transient risk factors such as surgery or trauma display altered fibrin variables.

Other Diseases

Sjøland et al reported alterations in fibrin properties in 22 patients on chronic peritoneal dialysis. Patients with end-stage renal disease had fibrin clots that were less permeable and resistant to fibrinolysis. Similar alterations in fibrin properties have been shown in end-stage renal disease patients on chronic hemodialysis. During a 3-year follow-up,
clots made from baseline plasma taken from patients who died of cardiovascular causes were significantly less permeable and lysed less efficiently than those from plasma of the remaining patients, indicating that altered fibrin properties may incur a worse prognosis in end-stage renal disease.

Patients with chronic obstructive pulmonary disease displayed unfavorable, compact, and poorly lysable fibrin structure, which could contribute to an increased risk of thrombotic events. Clot permeability and lysis time in chronic obstructive pulmonary disease patients were associated with CRP, a marker of inflammation, which was a stronger predictor for fibrin structure in this study than fibrinogen concentration. CRP binds to fibrinogen and thus may modify fibrin formation, although the mechanisms underlying such fibrinogen modification are unknown. In patients with advanced CAD, despite the presence of several clot-modifying risk factors, ie, diabetes, elevated CRP was associated with the formation of denser fibrin and resistance to lysis. Rheumatoid arthritis is another example of a chronic inflammatory disease with a high risk of MI, stroke, and VTE that associates with the formation of dense and poorly lysable clots. It is unclear whether effective therapy of rheumatoid arthritis associated with a marked reduction in CRP leads to improved fibrin characteristics.

Concluding Remarks
Fibrin clot structure and function are determined by genetic and environmental factors, including cigarette smoking, inflammatory status, hyperglycemia, oxidative stress, and elevated Hcy levels. Atherothrombotic vascular disease and VTE represent a major cause of morbidity and mortality worldwide. Growing evidence supports the concept that fibrin characteristics may represent a novel risk factor for arterial and VTE.

The associations between thrombosis and fibrin properties are reported largely in case-control and cohort studies. Drugs effective in the cardiovascular prevention, particularly aspirin and statins, can improve fibrin properties. It remains to be elucidated whether fibrin properties are vascular bed-specific and to what extent shear stress alters fibrin architecture. Relative contributions of cellular and soluble factors modulating fibrin formation in various diseases are also unknown. Prospective studies with long-term follow-up are required to investigate whether fibrin parameters can predict an increased risk for thromboembolic events in the general population and also in subjects with arterial or venous disease. Furthermore, the potential impact of prothrombotic fibrin phenotypes on progression of atherosclerosis and mechanisms involved in this process is also of interest.

Many of the mechanisms that determine fibrin structure remain to be elucidated. For instance, the mechanisms by which twisting, interconnected fibers and fiber bundles are formed from initial, small protofibrils are only beginning to be understood. Once detailed mechanisms have been determined, strategies to modulate fibrin structure with new, specific agents may be developed and their role in thrombosis explored using in vitro and in vivo experimentation, followed by first-in-human studies. The relationships between fibrin viscoelastic properties, interactions with cells (platelets, erythrocytes, and leukocytes), blood flow, and thrombus stability are other areas for future study. Knowledge of potential mechanisms involved in clot embolization is limited. Basic mechanisms that determine fibrin structure are only beginning to be understood, and many studies consistently report on altered fibrin structure in thrombosis. These findings hold the promise of future developments of new strategies for the treatment of thrombosis that remain undressed with current anticoagulants and thrombolitics.

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References


111. He S, Blomback M, Bark N, Johnsson H, Wallen NH. The direct thrombin inhibitors (argatroban, bivalirudin and lepirudin) and the indirect Xa-inhibitor (danaparoid) increase fibrin network porosity and thus facilitate fibrinolysis. *Thromb Haemost.* 2010;103:1076–1084.
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