Thrombosis: theoretical considerations

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ABSTRACT The blood coagulation process is initiated in response to vascular injury and results in either hemostasis or thrombosis. The process can be divided conceptually into separate steps including initiation, propagation, termination, elimination, and repair. Concise descriptions of each of these processes are provided in the present review together with an attempt to integrate these processes at a conceptual level so as to avoid the unfortunate tendency to apply linear logic to the complex temporal interplay among the various processes in the blood coagulation system.

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INTRODUCTION

Coincident with the development of a vascular system for the distribution and removal of nutrients and wastes, there has evolved a system designed to maintain the integrity of the vasculature to prevent leakage. This system simultaneously maintains blood in a fluid state such that leak prevention occurs only where a perforating vascular injury causes it to be required. The vascular hemostatic system is thus in a constant balance, providing leak-preventing solid plugs where appropriate while maintaining fluid throughout the vast architecture of the vasculature.

Clinical literature differentiates between the process of leak-preventing hemostasis, required when the vessel is challenged by a perforating injury and a desirable outcome, from the life-threatening process of thrombosis, in which a blood-clotting obstruction within the lumen of the vessel denies organs of their appropriate metabolic support (1). The system is complex, involving the vasculature itself, formed elements within the blood, and soluble proteins in the blood plasma. These elements cooperate such that the blood coagulation system is activated only where vascular damage requires clot formation.

The origin of vascular damage can be mechanical, chemical, or biological. Regardless of the initiating event, endothelial cell damage will elicit localized blood coagulation, which is followed by fibrinolysis and tissue repair. These processes probably cycle continuously without noticeable physiologic sequence associated with either significant blood loss or vascular occlusion. The various processes associated with the response to vascular injury include initiation, propagation, termination, elimination, and repair. These processes do not progress linearly and are highly intertwined.

BLOOD VESSELS

The lining of the vascular system is composed of a single layer of endothelial cells that constitutively display anticoagulant properties. These cells secrete prostacyclin, which is an antiaggregation agent toward platelets, and have on their luminal surfaces various defenses against coagulation (2). These defenses are both passive (the heparan sulfate anticoagulant system, which interacts with antithrombin III) and active (the thrombin-thrombomodulin system, which activates protein C anticoagulant systems) (3, 4). Endothelial cells can also be a clot-promoting surface when stimulated with certain types of cytokines (5). Under ordinary circumstances, endothelial cells are primarily anticoagulant in nature.

The architecture surrounding endothelial cells is a complex array of connective tissue and musclelike cells that control vascular tone (6). These cells, ordinarily not in contact with fluid blood, can provide a potent procoagulant activity in the form of tissue factor and surrounding cell membrane, which can initiate the blood coagulation system, leading to thrombin formation (7). In the subendothelial tissues is a complex array of matrix molecules, including collagens, von Willebrand factor, and elastin, which are targets for platelet receptors and can stimulate platelet adhesion and aggregation (8). The endothelial cells are also profibrinolytic, secreting plasminogen activators (tissue plasminogen activator and urokinase), which can stimulate plasma plasminogen activation and lead to dissolution of a fibrin clot (9).

In many respects most of the reactions that lead to clot formation and dissolution can be considered to be binary. The blood, in transit, supplies one element required for the reaction while the vascular or extravascular tissue provides the other. In the procoagulant response, tissue factor, resulting from the disruption of the endothelium, provides the activator required to initiate the plasma clotting system; the vascular matrix connective tissue components stimulate the adherence and aggregation of the blood platelet. Similarly, the anticoagulant processes are also binary: the plasma-derived antithrombin III system requires vascular heparan sulfate on the surface of the

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endothelium to provide its potent anticoagulant effect, and the thrombin-thrombomodulin anticoagulant system requires $\alpha$-thrombin and protein C derived from plasma and thrombomodulin presented on the surface of the endothelium. The fibrinolytic system is also a binary system, using plasminogen from blood plasma and a proteolytic activator secreted by the endothelial cell.

The blood coagulation and fibrinolysis systems must be under the regional control of the vasculature. Hence, it is appropriate that each of the regulatory systems involved in blood fluidity maintenance are binary in nature, involving elements of the fluid component and elements derived from the regionally defined architecture.

**Initiation**

 Initiation of the blood clotting process occurs because of a perturbation associated with cells of the blood vessel wall or with a circulating blood cell. The desirable process of hemostasis occurring as a consequence of vascular perforation has as its analogue the undesirable thrombotic event associated with a vascular obstruction and the formation of an internal blockade. About one-third of all individuals on this planet succumb to events associated with the latter process in the form of a myocardial infarction or stroke, generally associated with the vascular damage contributed by atherosclerosis and the damage-repair processes associated with its sequelae. The contributions of endothelial damage cannot presently be separated from the contributions of the coagulation-platelet system in the inception and propagation of the atherosclerotic lesion and the ultimate triggering of an occlusive thrombus. The products derived from a coagulation event are relevant to the architectural changes associated with the growth of an atherosclerotic lesion. Furthermore, the initiation of thrombus formation is mediated not only by the regulatory events associated with the endothelium and its surrounding tissue, but also by the status of the blood coagulation system, the anticoagulant system, and the fibrinolytic system.

Circulating platelets display on their surfaces passive and activation-dependent receptors, which can interact with many agonists, including small molecules, plasma proteins, and proteins from the extracellular matrix (Figure 1). Receptor-mediated interactions lead to a complex series of intercellular activation events, which lead to extrusion of multiple players that contribute to the overall hemostatic response. Receptor-mediated interactions lead to platelet adhesion and trigger the aggregation of blood platelets in the region of vascular damage.

One of the most important players in this process is the glycoprotein IIb-IX complex, which interacts with von Willebrand factor multimers associated with the vascular matrix and is associated with platelet adherence to sites of damage (11).

![FIGURE 1. Unactivated and activated platelets. Platelet activation is initiated by receptors on the platelet surface that are either constitutively expressed (白色) or expressed only after platelet activation itself (黑). Some constitutively expressed receptors transmit a signal across the membrane on receptor occupancy, others do not appear to carry out the signal transduction process. Both thrombin and collagen interact with receptors on the platelet membrane surface that can subsequently transmit the activation signal across the platelet membrane. The fibrinogen receptor on the platelet [glycoprotein (GP) IIb-IIIa] is quiescent in the resting state of the cell and expressed only after platelet activation. The von Willebrand factor (vWF) receptor (GP IIb-IX) is constitutively expressed on the unactivated and activated platelets. This receptor does not appear to transmit platelet activation signal response across the platelet membrane. Reprinted with permission from JB Lippincott Company (10).](https://academic.oup.com/ajcn/article-abstract/65/5/1657S/4655619)
Persons with von Willebrand disease have reduced or abnormal synthesis of von Willebrand factor and thus abnormal platelet adherence (12). In the alternative congenital syndrome Bernard Soulier disease, the expression of defective glycoprotein Ib-IX complexes or their absence produces similar pathology (13). Glycoprotein Ia-IIa complexes are expressed constitutively on the platelet surface and interact with matrix collagen leading to triggering of the platelet activation cycle (14). Other receptors present on the platelet surface interact with thrombin, ADP, serotonin, arachidonic acid, and various other agonists that can trigger the platelet activation release-aggregation cycle (15).

Platelet aggregation is a binary process involving the expression of the activation-dependent glycoprotein Ib-IIIa complex and its interaction with fibrinogen (16). Platelet activation also leads to the secretion of other effector molecules, which further influence the clot-related processes, including further platelet accumulation, neutralization of heparin (17), and secretion of factor V-Va (18) and fibrinogen (19). In addition, platelets are a rich source of platelet-derived growth factor and transforming growth factors, type β, which significantly influence the regulation of the nucleated cells in the region of the vascular injury (20, 21). Platelet activation also provides an anchoring point for the formation of the coagulation enzyme complexes that lead to expression of α-thrombin and ultimately fibrin at the wound site (22).

**Propagation**

The development of chelating anticoagulants led to extensive studies over the past century aimed at elucidating the principles by which plasma could form a fibrin clot. These laboratory studies, combined with known instances of bleeding pathology, led to most of our understanding of the reactions of the blood plasma coagulation process (Figure 2). However, the availability of in vitro plasma clotting analytic techniques has led to some confusion with respect to the relative significance of different reactions in the establishment of hemostasis.

Investigators recognized that when Ca²⁺ was resupplied with acidic phospholipids to citrated plasma, clotting would occur by an intrinsic pathway that involved a foreign surface and a plasma contact system. Biochemical studies showed that the contact system involved the zymogens factor XII, prekallikrein, and the binding protein high-molecular-weight kininogen (HMWK). A catalyst formed by surface activation of these proteins led to the activation of factor XI, and the factor Xla complex activated factor IX to factor IXa. This defined in vitro plasma coagulation system was nonresponsive for plasmas obtained from persons with hemophilia B (factor IX deficiency) or hemophilia A (factor VIII deficiency) (23). Previous investigators noted that the addition of tissue homogenates and Ca²⁺ to plasma led to rapid clotting (24). In lab clotting tests, this tissue-dependent process (the extrinsic pathway of coagulation) is not sensitive to the defects of hemophilia A and hemophilia B (25).

The intrinsic and extrinsic pathways of blood coagulation have been taught to medical students for many generations. However, although coagulation defects can be clearly shown in vitro to be associated with all elements of the coagulation pathway presented in Figure 2, the bleeding pathology associated with various congenital defects is not explained by this organization of enzymatic complexes. Individuals who lack elements of the intrinsic pathway, factor XII, prekallikrein, and kinogen (HMWK) do not have a clinically discernible hemorrhagic defect. Persons who lack factor XI have a relatively mild form of hemophilia (26). However, defects in subsequent parts of the coagulation pathway leading to thrombin (IIa) have significant hemorrhagic defects that require replacement therapy.

Each of the essential complexes associated with bleeding pathology involves a vitamin K-dependent enzyme that is derived from a plasma zymogen, a cofactor protein, membrane, and Ca²⁺ (27). The vitamin K-dependent zymogens, associated with the presence of γ-carboxyglutamic acid (created in a posttranslational modification event during synthesis of these proteins), include prothrombin, factor VII, factor X, factor IX, and protein C (28). These proteins were identified because of their abundance (prothrombin) or congenital absence (factor X and factor IX). The identification of γ-carboxyglutamate led to the identification of protein C and subsequently of protein S, which are important in the anticoagulant system (29, 30). Protein C is activated to activated protein C by α-thrombin complexed with thrombomodulin on the endothelial cell membrane (31).

The vitamin K-dependent zymogens are organized via multiple domain elements (Figure 3), including the γ-carboxyglutamate domain at the amino terminus and a serine protease domain at the carboxyl terminus (33). The intermediate domains are either composed of kringle, in the case of prothrombin, or epidermal growth factor (EGF) domains, in the case of

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**FIGURE 2.** Multiprotein complexes of the coagulation cascade, presented as complex enzymes that involve a serine protease, one or more cofactor proteins, divalent cations, and a surface. For activity expression of all the complexes, except the contact pathway (factor XII complex), an active enzyme is required. For the contact pathway, activated factor XII (factor XIIa) is generated as a consequence of binding to a surface. Reciprocal activations involving kallikrein and factor XIIa lead to the generation of substantial amounts of factor XIIa that activate factor XI to factor Xa. All the other complexes involve a phospholipid membrane surface and a cofactor protein acting in concert with a serine protease to convert the appropriate zymogen substrate to its respective product. HMWK, high molecular weight; PC, protein C. Reprinted with permission from JB Lippincott Company (10).
FIGURE 3. General structure of the vitamin K–dependent proteins. The approximate locations of the $\gamma$-carboxyglutamic acid residues ($\gamma$-Glu) and the domain containing epidermal growth factor–like structures (EGF domain) or kringle (K) type structures as well as the activation peptide (AP) are indicated within the general structure. Reprinted with permission from Elsevier Science (32).

Conversely, at low concentrations of tissue factor–factor VIIa, the principal mechanism by which factor Xa is generated occurs through the intrinsic factor Xase (factor VIIIa–factor IXa). Thus, the pathway kinetics depend largely on the available concentration of tissue factor–membrane that can support the reaction (42, 43).

The astute observer will recognize that factors VIIa and Va are derived from procofactors that require catalytic cleavage for their formation. The enzymes responsible for these activations are factor Xa–phospholipid and thrombin. The coagulation system thus is somewhat of a chicken-and-egg system in which one must first have enzyme to generate enzyme. The initial low levels of activation can provide extraordinary feedback amplification loops that lead to the explosive generation of the protein enzyme thrombin (44).

Schematic representations of the vitamin K–dependent enzyme procoagulant complexes and the anticoagulant thrombin-thrombomodulin complex are presented in Figure 4. This figure also emphasizes the importance of the membrane as the assembly point for each of these enzymatic complexes. This membrane can be provided by mechanical damage to the vasculature, various agonist-responsive cells, or both. Although each of the enzyme complexes exhibits discrete substrate and proteolytic specificity, the complexes have several common features: they are functionally analogous, with structurally homologous constituents; they exhibit similar requirements for assembly and activity; and the complex assembly leads to a significant enhancement (105- to 106-fold) for the localized catalytic rate of activation of the particular substrate.

The principal regulatory events associated with the formation of the vitamin K–dependent enzymatic complexes include the following: the conversion of a vitamin K–dependent zymogen from the plasma to a serine protease; the proteolytic activation of a plasma-derived procofactor to an active cofactor in the case of factors V and VIII or the membrane expression or presentation of an integral membrane cofactor, tissue factor, or thrombomodulin; the presentation of the appropriate phospholipid-like membrane surface that can accommodate the protein–protein and protein-membrane binding interactions essential for enzyme formation and substrate activation.

A computer simulation describing the nature of the response involving the procoagulant complexes in thrombin generation is illustrated in Figure 5. This is a representation of the thrombin generation rates when all proteins are present at their plasma concentrations and the tissue factor concentration is changed from 5 pmol/L to 5 nmol/L (43). The form of each of these thrombin-generation curves is the same; thrombin generation occurs vigorously after a significant lag period in which little thrombin is generated. The lag is principally associated with tissue factor–factor VIIa concentration. During the lag interval small amounts of enzyme (factor Xa and thrombin) generation lead to quantitative activation of the procofactors V and VIII to the cofactors Va and VIIa. Once the lag phase is complete, the thrombin generation rate is virtually independent of the tissue factor initiator concentration.

Thrombin, once generated, has numerous substrates in the biological milieu and in many respects can be considered to be a hormone. From the perspective of the coagulation reactions, however, the most important substrates are platelets, fibrinogen, and factor XIII (27). Fibrinogen is an almost symmetrical, six-polypeptide-chain plasma protein composed of two $\alpha$
chains, two Bβ chains, and two γ chains (AαBβγ) (Figure 6). Thrombin cleaves off the A and B terminal peptides of the Aα and Bβ chains to give the fibrin product, which self-associates to the fibrin clot. Thrombin also participates in feedback activation of factors V, VIII, XI, and VII. In addition, thrombin can activate plasma factor XIII to its active form, factor XIIIa (44). Factor XIII is present in plasma and in platelets. In the active form factor XIIIa is a transglutaminase that can introduce covalent cross-links between selected glutamyl and lysyl side chain residues to form a clot covalently stabilized by isopeptide bonds. The thrombin activation of platelets involves an intriguing thrombin substrate-receptor interaction. A protein on the surface of the platelet serves as a substrate for thrombin. The product of thrombin cleavage interacts as a tethered ligand for a membrane receptor that stimulates platelet activation (45).

**TERMINATION REACTIONS**

The termination reactions of the blood clotting process are both constitutive and reactive. Two constitutive inhibitors are implicated by congenital or induced anticoagulant pathology. These include antithrombin III (AT-III) and the tissue factor pathway inhibitor (TFPI) (46, 47). AT-III is identified as an important player by pathology associated with congenital de-
fects in the molecule. TFPI was shown in animal intervention studies to be a physiologic regulator of coagulation (48). AT-III is a serine protease inhibitor (serpin), which combines with a serine protease target to form a stable covalent product between the inhibitor and the active site of the target enzyme. AT-III inhibits most of the proteases of the coagulation system; however, the most important inhibitor targets are probably α-thrombin and factor Xa. AT-III activity is regulated by the heparan sulfate present on the endothelial cell surface and is probably the major contributor in the clinical efficacy of heparin as an anticoagulant.

Two types of heparin–AT-III binding interactions have been observed. In one type of interaction, a specific pentasaccharide sequence binds to and activates AT-III (49). Heparin, a highly anionic molecule, also binds less specifically with an exosite present on some enzymes of the blood clotting system. In the inhibition of factor Xa, the principal influence of heparinoids is associated with their ability to bind to AT-III through the pentasaccharide sequence that stimulates the initiation of the reaction, thus making AT-III a better inhibitor of factor Xa. In contrast, the inhibition of α-thrombin by AT-III is influenced both by the specific pentasaccharide heparin interaction with AT-III and by the coordinated binding of AT-III and thrombin (through the heparin binding exosite) to the anionic heparin polymer.

TFPI is present at much lower concentrations than AT-III. It was originally called lipoprotein-associated coagulation inhibitor (LACI) and extrinsic pathway inhibitor (EPI). TFPI is a Kunitz-type inhibitor with three inhibitor domains. It has a bivalent interaction in the coagulation system. TFPI binds the factor VIIa–tissue factor–factor Xa ternary complex. This inhibitor has been hypothesized to be the major regulator of the initiation events of the blood clotting process, probably acting during the lag phase. In contrast, AT-III, because of its abundance in plasma, most likely participates as a scavenger, removing excess circulating enzyme from plasma.

In addition to the constitutive inhibitors, the reactive dynamic inhibition system is clearly important in the regulation of coagulation. This reaction involves the association of α-thrombin derived from clotting with endothelial cell expressed thrombomodulin to form the protein C activator complex protein Case (Figure 4). The extent of the activator complex concentration depends both on the constitutively expressed thrombomodulin on the vascular endothelium and the amount of circulating thrombin made available as a consequence of a clotting reaction. The activated protein C product, once generated, serves an anticoagulant function by inactivating factors Va and VIIIa by selective peptide bond cleavage (50, 51). In both instances, the activation reactions depend on the presentation of an appropriate membrane surface. Endothelial cells and platelets both support this reaction; however, the relative contribution of each in the anticoagulant process is not known.

ELIMINATION

The formation of a fibrin clot is, presumably, a temporary, emergency measure devised by nature to prevent immediate blood loss. The temporary clot structure must subsequently be dismantled to permit formation of a more stable and suitable vascular architecture where the damage and clotting has occurred. The clot dismantling process is principally a consequence of the activation of plasma plasminogen to plasmin by two enzymes, single-chain urokinase (SCUPA) and tissue plasminogen activator (TPA) (52, 53). These two proteins are secreted as active enzymes from cells and do not absolutely require proteolysis to display protease-active sites. However, proteolytic modification of both of these proteins enhances their activities.

Plasminogen, TPA, and UPA all interact with various receptors, a variety of matrix components, and fibrin, leading to localized plasminogen activation (53, 54). The best known of these receptor interactions is associated with the colocalization of both plasminogen and TPA in fibrin clots; however, other matrix constituents (eg, collagen) can also lead to colocalization and activation of plasminogen (55). Agents that can potentially interfere with these colocalization processes can alter the fibrinolytic process.

The fibrinolytic process is highly regulated by both endothelial cell–derived and plasma-derived agents. Plasminogen activator inhibitor 1 inhibits TPA and SCUPA. Plasma-derived α-2 antiplasmin, a serine protease inhibitor, will rapidly and specifically inactivate plasmin. The fibrin clot is dissolved by plasmin-selective cleavages in the fibrillar regions between the globular domains in the fibrin molecule producing soluble products (Figure 7).

REPAIR

The ultimate repair processes of the vascular wall involve the generation of new connective tissue and matrix components derived from endothelial and smooth muscle cell populations. Transforming growth factor β and platelet-derived growth factor derived from local platelet activation are significant in this process, which ideally leads to a stable continuous vascular and anticoagulant architecture.

However, the vascular repair probably does not provide architecture identical to that existing before damage occurred. In general, the repair process leads to an overexpression of local cells and cellular processes in many respects similar to superficial scar tissue (56).

![FIGURE 7. Plasmin cleavage of fibrinogen and fibrin. Plasmin cleavage of fibrin and of non-cross-linked fibrin gives rise to solubilized products that consist primarily of the D and E domains of the initial fibrinogen molecule. When cross-linked fibrin (see Figure 6) is cleaved by plasmin, cross-linked D domains are also observed as products. Reprinted with permission from JB Lippincott Company (10).](https://academic.oup.com/ajcn/article-abstract/65/5/1657S/4655619)
LIPID INVOLVEMENT IN THE PROCESSES OF HEMOSTASIS AND THROMBOSIS

The associations, described in the literature, between dietary lipids and thrombosis-atherosclerosis are both extensive and confusing. Epidemiologic and direct intervention studies show that atherosclerotic lesions are clearly related to lipid and cholesterol. Thus, to the degree that the atherosclerotic lesion serves as a point of injury and thus a focus of response by the coagulation system, lipids are related to the coagulation process. How dietary lipids may directly influence the association with coagulation processes and its regulation is unclear. Because of the extensive interplay between the coagulation system and the arterial vascular damage process, some investigators have coined the term atherothrombosis. This term is somewhat useful in that it sums up the relation between the damage and repair processes and includes the clot-based occlusive events associated with thrombosis.

All of the procoagulant and anticoagulant vitamin K-dependent enzymatic complexes involved in coagulation and its control are essentially membrane dependent. In the absence of a membrane, the vitamin K-dependent complexes cannot form and cannot express their activities. Potential contributors of membrane to coagulation and anticoagulation reactions include endothelial cells, macrophages, platelets, and lipoproteins present in blood. Lipoproteins are also vehicles associated with the plasma transport of TFPI. Lipoprotein(a), the vehicle containing the plasminogen analog apolipoprotein(a), has been implicated as a potential regulator of the fibrinolytic process (57). Various literature reports suggest that dietary lipids can influence the state of vascular and blood cell activation, extent of damage of the vascular architecture, extent of activation of the coagulation system, state of the anticoagulant system of the blood clotting system, regulation of the fibrinolytic system at the level of plasminogen activation, and fibrinolytic activator and fibrinolytic secretion.

Scattered evidence exists for dietary lipid involvement in virtually all of these processes. Very-low-density lipoprotein (VLDL) was reported to bind factor Xa and participate in the coagulation reaction (58). Factor VII, identified as a risk factor in certain coagulation epidemiologic studies in terms of both antigen and activity, was reported to be increased in hyperlipidemia (59, 60). VLDL was also reported to bind factor VII (61), whereas lipoproteins were reported to inhibit factor Xa both in the presence and absence of TFPI (62). Oxidized LDL, significantly implicated as an atherogenic molecule, appears to enhance the induction of tissue factor by lipopolysaccharide in monocytes (63). Epidemiologic studies noted relation among triacylglycerols, low values of high-density-lipoprotein cholesterol, reduced glucose tolerance, hyperlipidemia, obesity, low physical activity, reduced fibrinolytic capacity, and increased factor VII concentrations (64). Dietary lipids may also influence endothelial cell tissue factor when combined with an inflammatory agent such as lipopolysaccharide (65). Indirect effects of dietary lipids can also possibly influence the absorption of vitamin K and anticoagulants (66).

SUMMARY

Associations likely exist between dietary lipids and some elements of thrombotic risks. However, at present it is difficult to identify explicit causal relations between events associated with blood clotting and dietary lipids per se. It is also difficult to discern whether lipids and lipoproteins have primary involvement in the initiation, propagation, termination, elimination, and repair processes or whether these processes are altered only as a consequence secondary to atherosclerotic injury associated with dietary lipids.

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