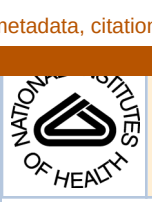

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Elevated prothrombin promotes venous, but not arterial, thrombosis in mice

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Abstract

Objective—Individuals with elevated prothrombin, including those with the prothrombin G20210A mutation, have increased risk of venous thrombosis. Although these individuals do not have increased circulating prothrombotic biomarkers, their plasma demonstrates increased tissue factor-dependent thrombin generation *in vitro*. The objectives of this study were to determine the pathologic role of elevated prothrombin in venous and arterial thrombosis *in vivo*, and distinguish thrombogenic mechanisms in these vessels.

Approach and results—Prothrombin was infused into mice to raise circulating levels. Venous thrombosis was induced by electrolytic stimulus to the femoral vein or inferior vena cava ligation. Arterial thrombosis was induced by electrolytic stimulus or ferric chloride application to the carotid artery. Mice infused with prothrombin demonstrated increased tissue factor-triggered thrombin generation measured *ex vivo,* but did not have increased circulating prothrombotic biomarkers in the absence of vessel injury. Following venous injury, elevated prothrombin increased thrombin generation and the fibrin accumulation rate and total amount of fibrin ~3-fold, producing extended thrombi with increased mass. However, elevated prothrombin did not accelerate platelet accumulation, increase the fibrin accumulation rate, or shorten the vessel occlusion time following arterial injury.

Conclusions—These findings reconcile previously discordant findings regarding thrombin generation in hyperprothrombinemic individuals measured *ex vivo* and *in vitro*, and show elevated prothrombin promotes venous, but not arterial, thrombosis *in vivo*.

INTRODUCTION

Arterial thrombosis and venous thrombosis/thromboembolism are traditionally regarded as distinct diseases with respect to their epidemiology and treatment strategies (reviewed in[1,2]). The presence of certain non-overlapping risk factors suggests that distinct features in the arterial and venous environments confer differential pathophysiology. Venous thrombosis is often associated with acquired or inherited plasma hypercoagulability and is thought to be

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Disclosure

The authors declare no competing financial interests.

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triggered by expression of cell adhesion molecules and procoagulant activity on intact endothelium in low shear. In contrast, arterial thrombosis is typically associated with atherosclerotic plaque rupture and exposure of subendothelial cells and highly procoagulant material (tissue factor [TF] and collagen) to blood in high shear. Consequently, venous thrombi are high in erythrocyte and fibrin content; whereas, arterial thrombi are platelet-rich. Treatment strategies to minimize venous thrombosis/thromboembolism and arterial thrombosis (anticoagulants and platelet antagonists, respectively) have reduced efficacy *vice versa*[1–3], supporting the premise that unique pathophysiological mechanisms promote thrombosis in veins and arteries.

Individuals with elevated prothrombin (hyperprothrombinemia), including those with the G20210A mutation in the prothrombin 3′-untranslated region[4,5], have ~3-fold increased risk for venous thrombosis. In particular, the G20210A mutation is associated with ~115–170% of normal prothrombin activity levels.[4–6] This mutation is present in 1–4% of the general European population[7], making it the 2nd most common genetic risk factor for venous thrombosis in Caucasians. In contrast, association of either elevated prothrombin or the G20210A mutation with arterial thrombosis is unclear.[8,9] Although the G20210A mutation has been weakly-associated with arterial disease[10] including coronary heart disease[11], ischemic stroke[12], and risk of myocardial infarction in young women[13] and men[14], other investigations have failed to support these findings in patients with cerebral ischemia[15,16], myocardial infarction[17], or general arterial events[18]. Patient heterogeneity may reconcile differences between these studies since arterial risk is increased in patients with additional risk factors (e.g., smoking, hypertension, diabetes and/or obesity)[13,14]; however, the independent association between elevated prothrombin and arterial thrombosis is difficult to discern in a human cohort and remains unresolved.

The operant pathological mechanisms of hyperprothrombinemia in either venous or arterial vascular beds are also unknown. Individuals with elevated prothrombin do not have increased circulating prothrombin fragment 1.2 levels[6], suggesting thrombosis does not result from constitutive activation of coagulation. However, *in vitro* studies show that following coagulation activation, high prothrombin levels increase thrombin generation[19–22], induce activated protein C resistance[23], and promote formation of abnormal fibrin networks[19] in clots that resist fibrinolysis[23,24]. These findings suggest elevated prothrombin levels promote thrombosis; however, this effect has never been directly demonstrated *in vivo*.

The objectives of the present study were to define the prothrombotic role(s) of elevated prothrombin *in vivo* and distinguish pathologic mechanisms differentiating these effects in venous and arterial thrombosis. We found that elevated prothrombin increased plasma thrombin generation *ex vivo* following TF-dependent initiation of coagulation, but did not activate coagulation or increase baseline thrombin generation *in vivo* in the absence of overt vascular injury. Following vascular injury, elevated prothrombin increased *in vivo* thrombin generation, but did not increase the rate of platelet accumulation in either arterial or venous thrombi. Elevated prothrombin increased the rate of fibrin deposition and produced larger thrombi in models of venous thrombosis. In contrast, elevated prothrombin did not increase the rate of fibrin deposition or shorten the time to occlusion (TTO) in models of arterial thrombosis. These data are the first to show that elevated prothrombin levels directly promote venous thrombosis *in vivo*, and show elevated prothrombin has little to no independent contribution to arterial thrombosis in the absence of additional risk factors.

MATERIALS AND METHODS

Materials and methods are available in the Supplemental Materials.

RESULTS

Human prothrombin is active in murine plasma

The *in vivo* hyperprothrombinemia model was developed by infusing mice with human prothrombin. Human prothrombin and the thrombin B chain have 81.4% and 88.8% amino acid identity with murine prothrombin and thrombin, respectively, and highly-conserved substitutions in non-identical residues.[25] Human and mouse thrombin bind and cleave human and mouse fibrinogen[26], activate platelets to form aggregates with pseudopodia[27], bind murine thrombomodulin, and support activated protein C generation[28,29]. To assess the ability of human (pro)thrombin to support thrombin generation in murine plasma, murine plasma was spiked with vehicle or murine or human prothrombin to 200% (final, endogenous plus spiked human prothrombin) and thrombin generation was measured by calibrated automated thrombography in the absence and presence of 200 nM murine thrombomodulin. Thrombin generation peaks were 49.4±7.2, 82.4±13.8 and 90.4±5.2 nM (mean±SD, n=2–3) for plasma plus vehicle, plasma plus murine prothrombin, or plasma plus human prothrombin, respectively, and addition of murine thrombomodulin reduced the thrombin peaks by 73, 74, and 64%, respectively (Supplemental Figure I). These data demonstrate that human (pro)thrombin is compatible with the murine procoagulant and anticoagulant systems.

In the absence of vessel injury, elevated prothrombin does not activate coagulation

Platelet-poor plasma (PPP) from patients with elevated prothrombin demonstrates increased thrombin generation *ex vivo*[6,22]; however, these patients do not have higher circulating prothrombin fragment 1.2 compared to age-matched controls.[6] We hypothesized that elevated prothrombin does not independently cause thrombin generation in the absence of vascular injury, but increases thrombin generation following TF exposure. To test this hypothesis, we infused prothrombin (to 300% of normal levels; total prothrombin equals endogenous murine prothrombin plus infused human prothrombin) or vehicle (20 mM HEPES (pH 7.4)/150 mM NaCl (HBS), Control) into uninjured mice and measured prothrombin antigen by western blotting, thrombin generation *ex vivo* by calibrated automated thrombography, and thrombin-antithrombin complex (TAT) levels by ELISA 12 hours after infusion. Human prothrombin still circulated in mice 12 hours post-infusion and was strongly detected by the rabbit anti-human prothrombin antibody (Supplemental Figure II). Consistent with humans, thrombin generation was significantly elevated in PPP from prothrombin-infused mice compared to controls (49.5±2.1 *versus* 39.3±2.5 peak thrombin, respectively, mean±SEM, P<0.04) (Figure 1A) following initiation of coagulation *ex vivo*. However, also consistent with that seen in humans, circulating TAT levels in mice with elevated prothrombin, even at this high level, were not elevated compared to controls (2.4±1.4 *versus* 3.8±4.0 ng/mL, respectively, mean±SEM, P=0.26) (Figure 1B). These data reconcile outwardly discordant findings regarding thrombin generation in hyperprothrombinemic individuals measured *ex vivo* and *in vitro* by showing that elevated prothrombin does not increase baseline hemostatic “idling” in the absence of vascular injury, but augments thrombin generation following a procoagulant trigger.

Elevated prothrombin accelerates fibrin deposition and produces larger thrombi in venous thrombosis models

To characterize the effect of elevated prothrombin on venous thrombosis *in vivo*, we first triggered thrombosis in the murine femoral vein via electrolytic injury and used intravital fluorescence detection to characterize the temporal and spatial contributions of elevated prothrombin to thrombus formation. The electrolytic injury model induces mural thrombus formation via iron-mediated injury that causes early platelet accumulation followed by fibrin accumulation[30] (Figure 2A, Supplemental Videos I, II). Thrombus formation in this model

is reduced by heparin, consistent with the sensitivity of venous thrombosis to thrombin generation.[31] We tested 2 levels of prothrombin: 130% and 200% (final); these levels were chosen to approximate the mean and upper end of the pathophysiologic range. Neither prothrombin concentration significantly increased the rate or total amount of platelet accumulation in femoral vein thrombi (Figures 2B, 2D). However, the plasma prothrombin level showed a dose-dependent effect on fibrin accumulation in the vein. At 60 minutes, fibrin accumulation in mice infused to 130% and 200% prothrombin was 1.7- ($p<0.06$) and 3.5- ($p<0.002$) fold higher, respectively, than control mice (Figure 2C), and mice infused with 200% prothrombin exhibited a significantly (2.3-fold, $p=0.006$) increased fibrin accumulation rate than control mice (Figure 2D). Furthermore, in contrast to control thrombi that remained relatively localized to the thrombus induction site, thrombi in prothrombin-infused mice showed considerable downstream elongation of a mass containing both fibrin and platelets (Supplemental Videos I, II).

We also characterized the effect of elevated prothrombin on venous thrombosis in an inferior vena cava (IVC) ligation (stasis) model that triggers venous thrombosis via vessel distention, blood stasis, and dysfunction (exposure of vessel wall TF) of intact endothelium.[32] For both control and prothrombin-infused mice, circulating TATs were significantly higher in mice that underwent IVC ligation than in mice that did not (Figure 3A), demonstrating activation of coagulation following vessel ligation. Circulating prothrombin was spiked to 300% so that levels remained elevated for the duration of thrombus formation (12 hours). Following IVC ligation, TATs were more than 2-fold higher in prothrombin-infused mice compared to control mice (47.9±6.5 *versus* 21.2±4.5 ng/mL, respectively, mean±SEM, $p<0.009$, Figure 3A), suggesting elevated prothrombin augmented thrombin generation during venous thrombogenesis. Following IVC ligation, all prothrombin-infused and control mice developed thrombi within 12 hours. Similar to that seen in the electrolytic injury model, thrombi in prothrombin-infused mice were significantly larger than thrombi in control mice (27.6 (26.1–43.6) *versus* 22.6 (9.5–27.7) mg, respectively, median (range), $p=0.01$, Figure 3B), and in some mice extended into the iliac branches. Together, these data show that elevated prothrombin augments venous thrombus formation *in vivo* by increasing thrombin generation and intravascular fibrin deposition.

Elevated prothrombin has little to no effect on the TTO or rate of platelet accumulation in arterial thrombosis models

We then used the electrolytic injury and real-time fluorescence detection method to measure the kinetics of platelet and fibrin accumulation in control and prothrombin-infused mice during arterial thrombosis (Figures 4A–4D, Supplemental Videos III, IV). Platelet accumulation in the artery was 3.2-fold faster ($p<0.003$) than that in the vein (Figure 4B, 4D vs 2B, 2D) and was complete within 10 minutes, consistent with the strongly platelet-dependent mechanism associated with arterial thrombosis. At 60 minutes, arterial thrombi also had 50% less fibrin deposition than venous thrombi (Figure 4C vs 2C), further illustrating mechanistic differences in thrombus formation in these two vessels. Arterial clots exhibited some embolization, indicated by the loss of mean fluorescence. As in the venous model, prothrombin levels were raised to 130 and 200% of normal (final). Neither prothrombin concentration significantly increased either the rate or total amount of platelet accumulation, or the rate of fibrin accumulation in carotid artery thrombi (Figures 4B–4D). Elevated prothrombin increased fibrin deposition at 60 minutes (~2-fold, Figure 4C), but this difference did not reach statistical significance ($P<0.085$).

We then tested the effect of elevated prothrombin in a second model of arterial thrombosis. The $FeCl_3$ application/carotid artery model triggers arterial thrombosis via generation of reactive oxygen species and exposure of collagen, resulting in a platelet-rich thrombus.[33–35]

Since neither prothrombin concentration significantly altered arterial thrombus formation in the electrolytic model, we only tested the higher concentration of prothrombin (infusing to 200%, final) in the $FeCl_3$/carotid artery model. Prothrombin infusion transiently elevated TAT levels (12.6±3.2 ng/mL), likely reflecting mild activation of coagulation from the venous infusion or trace (<0.004%) thrombin contamination in the prothrombin concentrate that was immediately inhibited by endogenous antithrombin (present in 1000-fold excess). This low thrombin level does not have any physiologic effects.[36,37] For both control and prothrombin-infused mice, circulating TATs were significantly higher in mice that underwent $FeCl_3$ injury than in mice that did not (Figure 4E), demonstrating activation of coagulation following $FeCl_3$ injury. Following $FeCl_3$ injury, TATs levels were significantly different between control and prothrombin-infused mice (13.9±1.7 *versus* 23.5±2.6, for control and prothrombin-infused mice, respectively, P<0.007, Figure 4E). However, the absolute increase in TAT levels over baseline was similar in both control and prothrombin-infused mice, suggesting arterial injury activated coagulation to a similar degree in both groups. Consistent with this observation, both control and prothrombin-infused mice developed stable, occlusive thrombi, and the time to vessel occlusion in prothrombin-infused mice was not different from controls (9.5 [4.3–40.0] *versus* 6.4 [4.5–8.5] minutes), respectively, median [range], Figure 4F). Thus, elevated prothrombin did not significantly increase platelet or fibrin accumulation during arterial thrombus formation or shorten the time to artery occlusion. Together, these data show that although elevated prothrombin promotes venous thrombus formation, it does not significantly augment arterial (platelet-dependent) thrombosis.

DISCUSSION

Elevated prothrombin is a well-established risk factor for venous thrombosis, but its relationship to arterial thrombosis is unclear. Using state-of-the-art *in vivo* models of venous thrombosis and arterial thrombosis, we show that elevated prothrombin did not increase baseline prothrombotic markers in unchallenged mice, but did increase thrombin generation following venous injury. The presence of elevated prothrombin did not accelerate intravascular platelet accumulation following either venous or arterial injury. In venous thrombosis models, mice with elevated prothrombin exhibited an increased rate and amount of fibrin accumulation, thrombus extension and formation of thrombi with increased mass. However, in arterial thrombosis models, elevated prothrombin slightly (non-significantly) increased the total amount of fibrin deposited, but did not increase the rate of fibrin accumulation or shorten the TTO. These findings suggest elevated prothrombin has little to no independent contribution to arterial thrombosis, and are the first to show that elevated prothrombin levels directly promote venous thrombosis *in vivo.*

The choice of murine thrombosis model for investigating human thrombosis has been the subject of considerable debate because many models fail to recapitulate key aspects of the arterial and venous thrombogenic processes.[38] An important strength of our study was the use of complementary arterial and venous models to delineate both kinetic processes and their consequences for thrombus composition. Complementary, integrated information from the two arterial models and two venous models reveals both common and vascular bed-specific processes operant in these vessels that are consistent with the histological appearance of arterial and venous thrombi isolated from humans. Arterial injury produced rapid platelet accumulation; whereas, venous injury resulted in slower thrombus formation with fibrin accumulation. We used models that produced both occlusive ($FeCl_3$/carotid and IVC) and non-occlusive (electrolytic injury) thrombi. Findings were consistent within vessels, but differed between arteries and veins, suggesting these models are sensitive to the unique physical and biochemical environments within the different vessels.

We detected both common and vascular bed-specific effects of elevated prothrombin on arterial and venous thrombosis. Elevated prothrombin significantly augmented endogenous thrombin generation in the venous model, but only slightly (non-significantly) in the arterial model (Figures 3A, 4E). Consistent with our findings, a recent study showed that *Apo*E$^{-/-}$ mice expressing half of the prothrombin level of wild type mice (*FII*$^{/+}$) are not protected from arterial thrombosis, supporting the conclusion that variation in the prothrombin level does not mediate arterial occlusion times.[39] Notably, although thrombin is a potent agonist for platelet activation, elevated prothrombin did not accelerate the rate of platelet accumulation in either arteries or veins (Figures 2D, 4D). These findings are consistent with our prior observation that 5% prothrombin is both necessary and sufficient to maximize platelet activation.[19] Consequently, elevated prothrombin did not accelerate arterial occlusion, a platelet-dominated process. In contrast, the increased thrombin generation significantly increased fibrin deposition and therefore, venous thrombosis, a fibrin-dominated process. We previously showed that elevated prothrombin also promotes the thrombin concentration-dependent formation of plasma clots with an abnormally dense fibrin network.[19] Although fibrin network structure is difficult to assess in thrombi in the presence of cells, combined, these results suggest that in veins, elevated prothrombin promotes thrombi with fibrin networks that have *both* increased mass and increased network density. Both properties are associated with increased clot stability *in vitro* and *in vivo,* and have been correlated with increased thrombosis risk.[40]

It is interesting that while elevated prothrombin did not accelerate arterial occlusion after $FeCl_3$ injury, elevated plasma factor VIII does[41–43]. Both elevated prothrombin and elevated factor VIII increase thrombin generation *in vitro*[19–21,43,44] and *in vivo* (Figure 3A and [41]). However, elevated factor VIII significantly shortens the lag time to platelet aggregation *in vitro*[41] and trends towards increased platelet accumulation *in vivo*[43]; whereas, elevated prothrombin did not significantly change the rate of platelet aggregation *in vitro*[19] or *in vivo* (Figures 2D, 4D). These data suggest elevated factor VIII modulates an early (amplification) phase of coagulation when thrombin levels are relatively low and platelet activation is taking place. Consequently, platelet-dependent arterial thrombosis models are sensitive to the effects of elevated factor VIII, but not to elevated prothrombin. This observation is consistent with the premise that procoagulant factors have complementary, but distinct, roles in different phases of coagulation.[45]

Previous studies on the association between elevated prothrombin and arterial thrombosis (myocardial infarction or ischemic stroke) have been inconsistent, showing either no or a modest relationship.[8–18] One explanation for these differences is that risk is present only in specific groups. Of interest are observations that relative risk increases when another identifiable cardiovascular risk factor is also present and appears higher than from either risk factor alone, suggesting an additive or synergistic interaction.[13,14] A strength of our murine model in which prothrombin levels were acutely elevated in healthy wild type mice is the clear absence of other risk factors. Nonetheless, the co-existence of additional known or unidentified risk factors may augment the positive associations detected in prior studies with human cohorts. For example, on an atherosclerosis-prone background (*Apo*E$^{-/-}$), chronic plasma hypercoagulability (TM$^{Pro/Pro}$) increases atherogenesis and plaque formation, both of which are associated with atherothrombosis, and reduced prothrombin levels attenuate atherosclerotic lesion formation.[39]

This study has potential limitations. First, we used human prothrombin to increase circulating levels in the mouse. However, published studies[25–29] as well as our data show human prothrombin is stable in murine circulation and participates in murine pro- and anticoagulant pathways. Moreover, the infusion strategy enabled us to precisely control the level of circulating prothrombin. Second, the infusion model used in these experiments does

not reflect pathologic effects that chronic exposure to elevated prothrombin could have on the vasculature. Atherosclerotic disease reflects chronic vascular injury with occurrences of acute injury (plaque disruption and TF exposure). However, a major strength of the infusion model is that it enabled us to isolate and investigate the immediate, direct effects of elevated prothrombin on thrombus formation. These data on acute effects will be critical for interpreting findings from mice with genetically-induced chronic plasma hypercoagulability (*e.g.*, factor V Leiden mice); comparison of short-term and long-term exposure to hypercoagulability is likely to reveal interesting mechanisms that predispose these individuals to thrombosis. Third, the thrombosis models we used differed in methodologic aspects, including anesthesia and analgesia protocols. However, the observation that elevated prothrombin exhibited consistent effects in each of the venous and each of the arterial models suggests the observed effects were not due to the methodologies, but to the prothrombin level, itself. Finally, the arterial and venous models used in this study were sensitive to thrombus formation, but did not reflect additional effects elevated prothrombin may have on thrombus stability. For example, although groups have demonstrated increased activation of the thrombin activatable fibrinolysis inhibitor in plasma with increased prothrombin, we did not evaluate the long-term resistance of thrombi to fibrinolysis.

In summary, our findings demonstrate that elevated prothrombin does not trigger endogenous thrombin generation in the absence of vascular injury, suggesting that in lieu of a signal that *initiates* coagulation, plasma hypercoagulability is not independently prothrombotic. These data suggest that increased coagulation biomarkers (*e.g.*, fragment 1.2 or TATs) indicate *vascular dysfunction* that, when coupled to additional plasma prothrombotic potential, promote thrombosis. Our findings further show that elevated prothrombin increases thrombin generation following vascular injury. Elevated prothrombin does not accelerate platelet activation in either the artery or the vein, but significantly increases the rate and amount of fibrin deposition following venous injury. These findings are consistent with findings that elevated prothrombin is associated with venous thrombosis in humans[4–6,46], but is only weakly associated with arterial thrombosis in the absence of other risk factors.[13–18,46] These results support the relevance of murine thrombosis models to studies of hypercoagulability-related thrombosis in humans. Integrating complementary *in vivo* models is a powerful approach to investigate the underlying mechanisms of hemostatic and thrombotic processes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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SIGNIFICANCE

Elevated plasma prothrombin, including that associated with the common G20210A prothrombin mutation, is associated with significantly increased thrombosis risk. Our findings demonstrate that elevated prothrombin does not trigger thrombin generation in the absence of vascular injury, suggesting that in lieu of a signal that *initiates* coagulation, plasma hypercoagulability does not independently causes thrombosis. However, following venous, but not arterial injury, elevated prothrombin accelerates fibrin accumulation and increases thrombus growth. These findings are the first to identify a causative mechanism by which elevated prothrombin promotes thrombosis *in vivo*. This study shows the importance of using integrated, complementary murine thrombosis models to understand hypercoagulability-related thrombosis in humans.

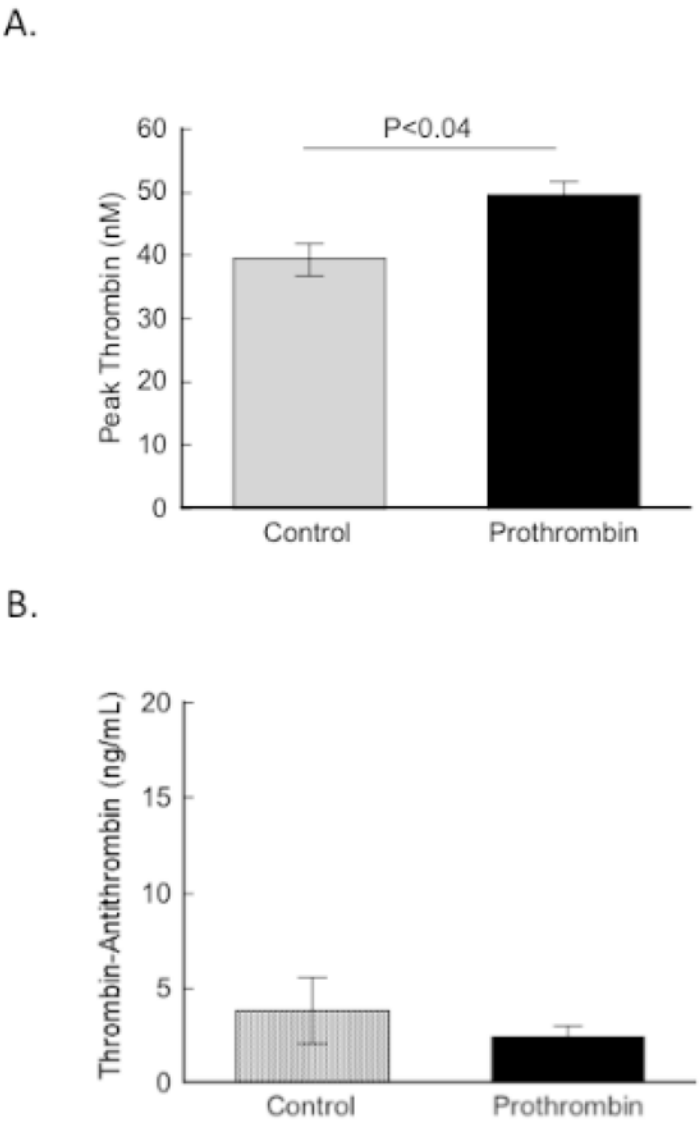

Figure 1. In the absence of vessel injury, elevated prothrombin does not activate coagulation

HBS (Control) or human prothrombin was infused into mice via tail vein injection to 300%, final (mouse plus human). Twelve hours after infusion, blood was drawn from the IVC into 3.2% sodium citrate and processed to PPP. A) PPP from mice infused with prothrombin (to 300%) or HBS (Control) was diluted 1:3 and coagulation was triggered by addition of TF/lipid. Thrombin generation was measured by calibrated automated thrombography (n=3/group). B) TAT levels were measured by ELISA (n=5/group). Bars show mean±SEM.

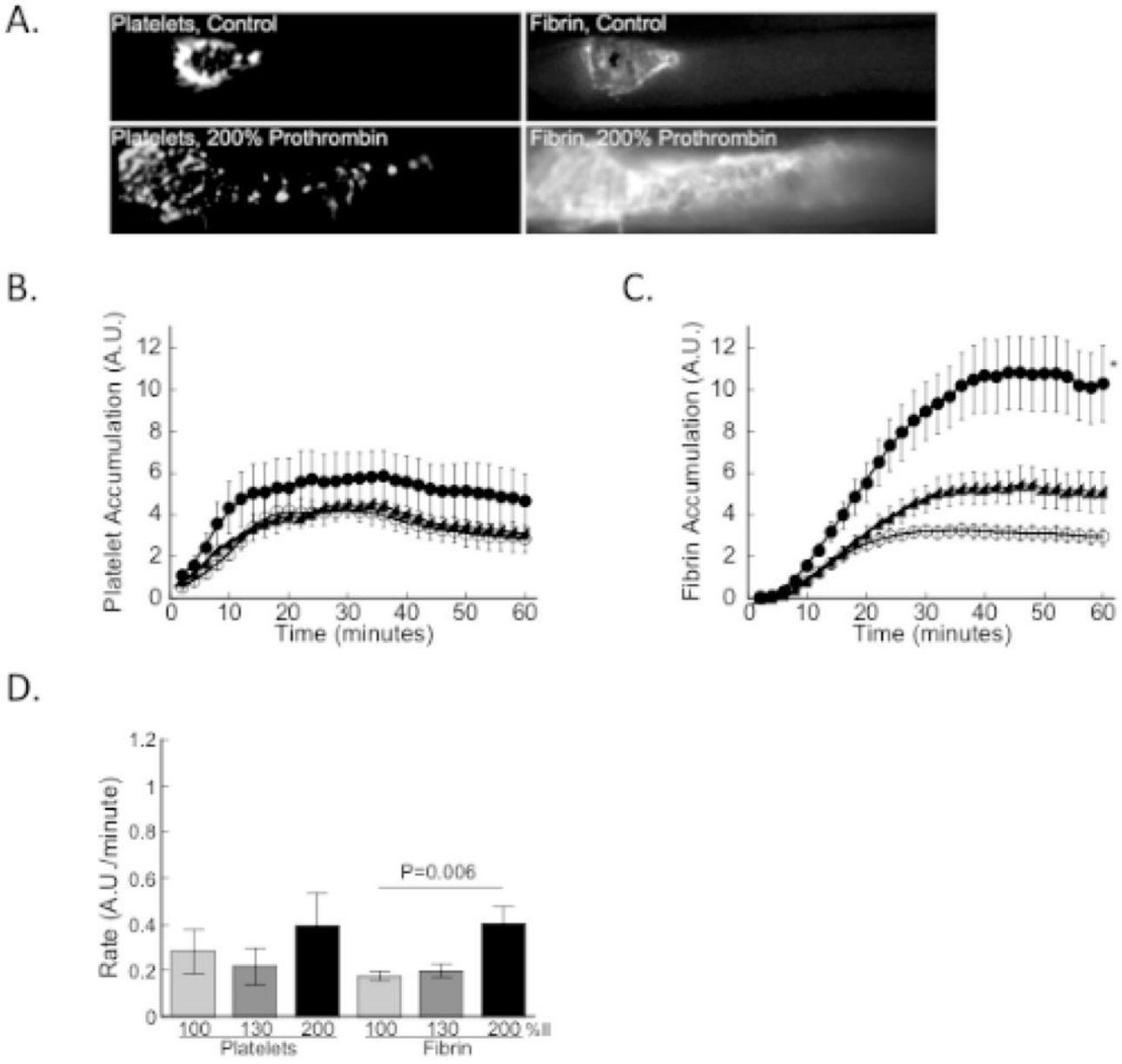

Figure 2. Elevated prothrombin increases the rate and extent of fibrin deposition following electrolytic injury to the femoral vein

Mice were infused with vehicle (control) or prothrombin to 200% of normal. Thrombosis was induced by electrolytic injury as described in Methods. A) Representative images of platelet and fibrin accumulation 60 minutes after induction of femoral vein thrombosis via application of electric current are shown. Flow is from left to right; field dimensions are 3.20 x 0.85 mm (width x height). B–C) Relative fluorescence intensity of platelet (B) and fibrin (C) accumulation following electrolytic injury to the femoral vein (mean±SEM, n=8/group, *P<0.002). Symbols are: control (open circles) and prothrombin-infused (closed triangles, 130%; closed circles, 200%). D) Maximum rate of platelet and fibrin accumulation during femoral vein thrombus formation following electrolytic injury (mean±SEM, n=8/group).

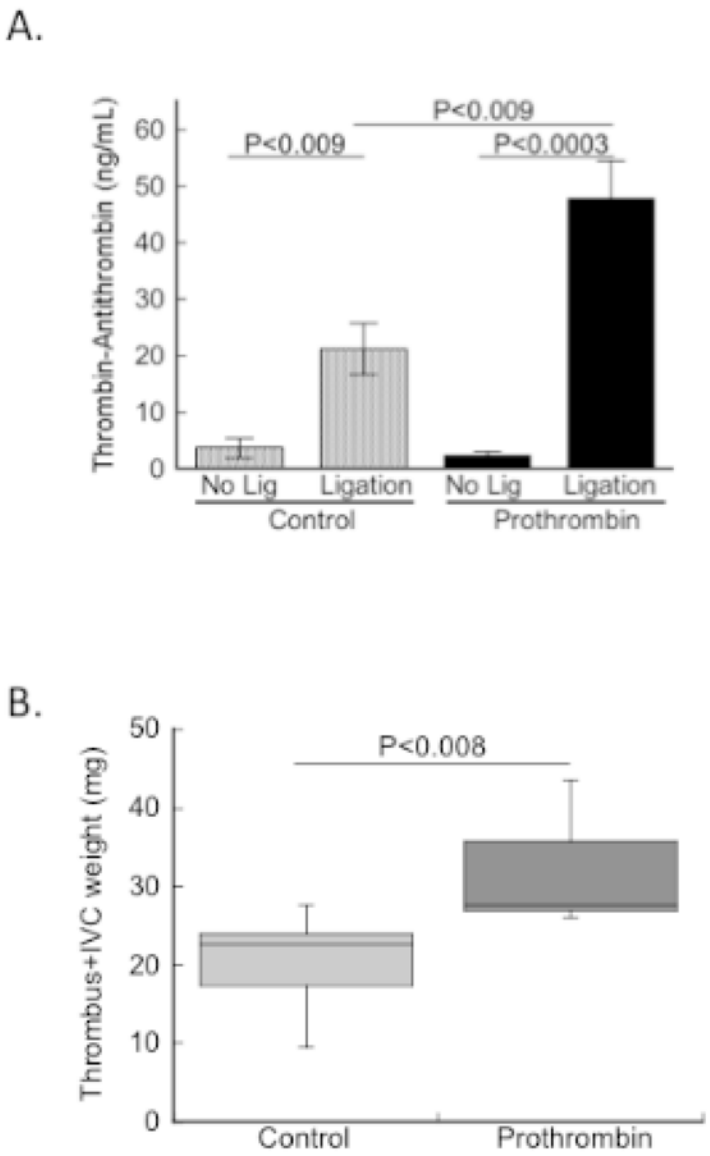

Figure 3. Elevated prothrombin produces larger venous thrombi by increasing thrombin generation following IVC ligation

A) IVC stasis was induced in mice infused with prothrombin (to 300%) or vehicle control. Twelve hours after ligation, blood was collected into citrate from just above the ligation site. TAT levels were measured from ligated (n=6–8/group) and uninjured mice (n=5/group) in parallel. Bars show mean±SEM. B) Twelve hours after ligation, the IVCs plus thrombi were excised and weighed. The box plot indicates medians and upper and lower quartiles (n=6–8/group).

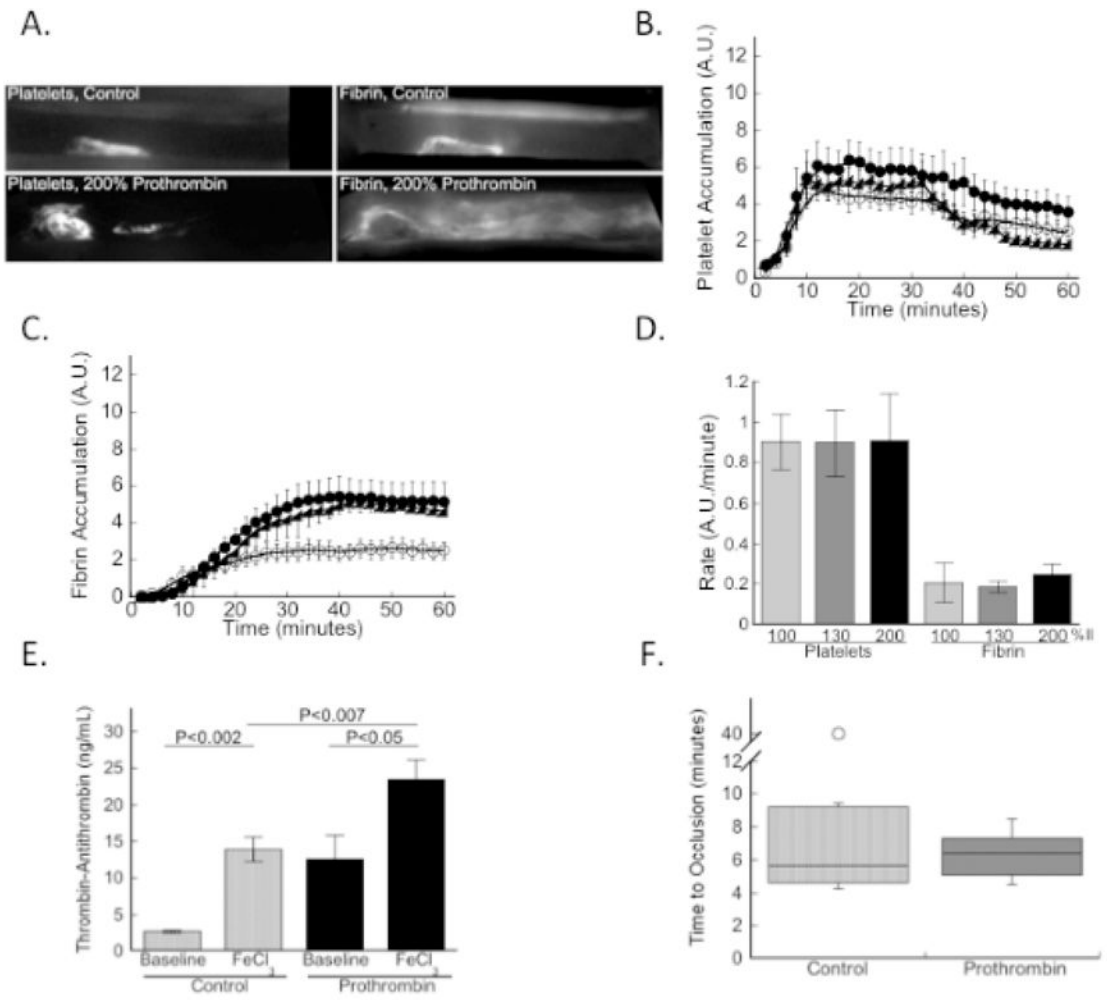

Figure 4. Elevated prothrombin does not increase thrombin generation or the rate of platelet or fibrin accumulation, and does not shorten the TTO in arterial injury models

A) Mice were infused with vehicle (Control) or prothrombin to 130 or 200% of normal. Thrombosis was induced by electrolytic injury as described in Methods. Representative images of platelet and fibrin accumulation 60 minutes after induction of carotid artery thrombosis via electrolytic injury are shown. Flow is from left to right; field dimensions are 3.20 x 0.85 mm (width x height). B–D) Relative fluorescence intensity of platelet (B) and fibrin (C) accumulation following electrolytic injury to the carotid artery (mean±SEM, n=7–8/group). Symbols are: control (open circles) and prothrombin-infused (closed triangles, 130%; closed circles, 200%). D) Maximum rate of platelet and fibrin accumulation during carotid artery thrombosis following electrolytic injury (mean±SEM, n=7–8/group). E) Mice were infused with vehicle (Control) or prothrombin to 200% of normal. Thrombosis was induced by 7.5% $FeCl_3$ application to the carotid artery for 2 minutes. Following thrombus formation, blood was collected from the IVC into citrate and processed to PPP. TAT levels were measured in $FeCl_3$-treated (n=7–10/group) and uninjured mice (n=4/group) in parallel. Bars show mean±SEM. F) Following $FeCl_3$ injury, the TTO was determined by flow probe. The box plot indicates medians and upper and lower quartiles (n=10/group); the open circle indicates one mouse that did not occlude.