

# Effects of Repeated Bouts of Exercise on the Hemostatic System

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## Abstract

Physical activity is beneficial for health, for example, by lowering the risk of cardiovascular events. However, vigorous exercise is associated with the occurrence of thromboembolic events and sudden cardiac death, in particular in untrained individuals. Whereas acute exercise is known to cause a hypercoagulable state, repeated exposure to (strenuous) exercise by means of training may actually condition the hemostatic response to exercise. To date, the effects of exercise training on blood coagulability and the underlying mechanisms have yet to be fully discerned. In this review, the authors provide an overview of existing literature on how training programs and training status influence hemostasis in healthy individuals. Furthermore, they present data of a pilot study in which we studied the effects of repetitive submaximal intensity cycling on procoagulant and anticoagulant processes. It is known that factor VIII (FVIII) and von Willebrand factor (VWF) increase after exercise, but we found that this increase in FVIII and VWF (antigen, propeptide, and VWF in active conformation) was smaller on each of three subsequent days, suggesting either adaptation of endothelial activation or exhaustion of endothelial VWF supplies. With respect to thrombin generation, elevated FVIII significantly increased the thrombin generation peak but not the endogenous thrombin potential. In contrast, platelet activation in terms of P-selectin expression after stimulation with protease-activated receptor-1 and glycoprotein VI agonists decreased after exercise and did not recover, indicating exhaustion of the platelet response to repetitive exercise.

## Keywords

- ▶ physical training
- ▶ repetitive exercise
- ▶ hemostasis
- ▶ coagulation
- ▶ fibrinolysis

Cardiovascular disease remains the leading cause of mortality and morbidity globally.<sup>1</sup> Regular exercise is essential for a healthy lifestyle, promoting cardiovascular health, as shown by many epidemiological and clinical studies.<sup>2–4</sup> Several studies have reported an association of increasing physical activity with reduced morbidity and mortality,<sup>5,6</sup> which can be explained by

benefits such as weight loss, lowered blood pressure, and improved lipid profiles and glucose metabolism.<sup>7–9</sup>

Regular physical activity is associated with the prevention of cardiovascular disease, with an effect similar in magnitude (~30%) to pharmacological strategies.<sup>10</sup> However, more physical exercise may not always be better. Indeed, the benefit of

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exercise is still a subject of debate that is referred to as the “exercise paradox.”<sup>11,12</sup> The debate is relevant because there are numerous reports of exercise-related thromboembolic events, such as myocardial infarction, ischemic stroke, and venous thrombosis,<sup>13,14</sup> and acute exercise is associated with a two- to threefold higher risk of sudden cardiac death.<sup>15,16</sup>

One crucial protagonist of atherosclerotic and atherothrombotic disease is the hemostatic system.<sup>17</sup> Exercise is known to exert a plethora of effects on hemostasis. These include increased levels of clotting factors (factor VIII [FVIII] and von Willebrand factor [VWF]), platelet count and reactivity, a shortening of the activated partial thromboplastin time (aPTT)<sup>18,19</sup> and increased thrombin generation (TG),<sup>20</sup> as well as increases in fibrinolytic markers (prothrombin fragments 1 + 2 and thrombin–antithrombin complexes).<sup>21,22</sup> Altogether, these changes result in a shift toward a transient hypercoagulable state,<sup>23</sup> that depends at least partly on exercise intensity.<sup>24</sup> Whereas moderate exercise enhances fibrinolytic activity, strenuous exercise (corresponding to 80–100% of the maximal heart rate) induces a more procoagulant state, causing an enhanced risk of thrombotic events, especially in untrained individuals.<sup>25–27</sup> Contrary to acute high-intensity exercise, regular training at moderate intensity is associated with a lower overall risk of adverse cardiovascular events.<sup>28,29</sup>

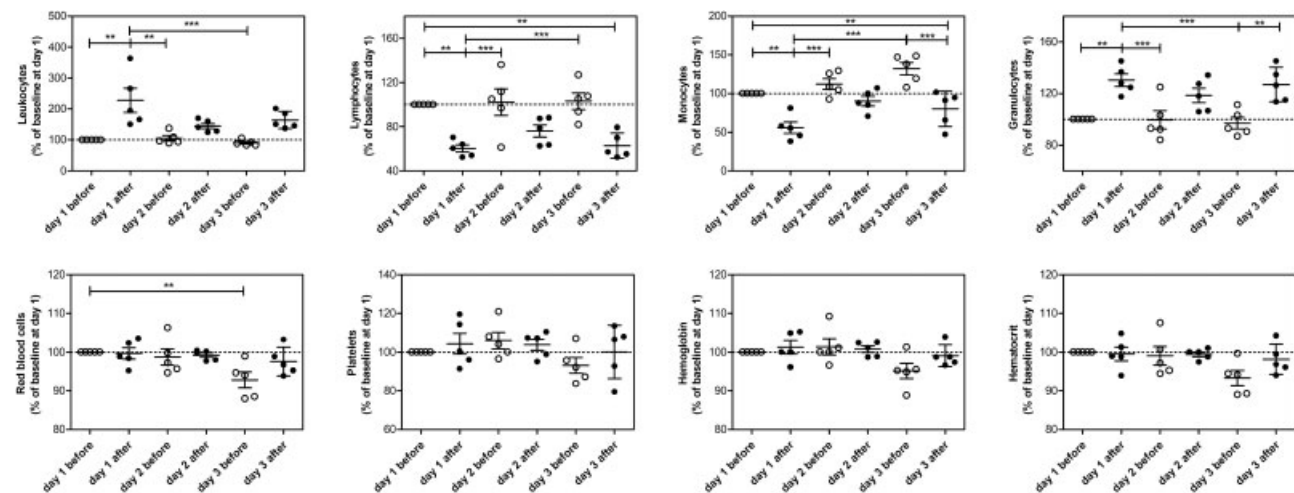
The effects of (strenuous) exercise on hemostasis have been reviewed extensively by others.<sup>23,26,30</sup> Many studies report on the effects of a single bout of vigorous physical activity (either of short or long duration, the latter mostly in marathons). However, it is not a single bout but long-term repeated exercise that results in improved physical fitness which is known to be beneficial for the cardiovascular system<sup>31,32</sup> and is related to reduced mortality.<sup>33,34</sup>

To date, the effects of repeated bouts of exercise on hemostasis and the underlying mechanisms have yet to be fully discerned, and data on this specific topic is lacking. Therefore, we recently performed a pilot study in which we studied whether repetitive strenuous cycling had an additive, exhaustive, or adaptive effect on changes in procoagulant and antic-

oagulant processes (see the ►Appendix). More data on the effects of training on these variables are becoming available. This review aims to provide insight of how repeated exercise influences hemostatic parameters (in healthy individuals), by placing it in the context of what is known on the hemostatic effects of training programs and improved training status. We defined “exercise training” as repeated exercise undertaken at a guided or prescribed intensity (either moderate or intensive) and frequency over a specific period of time.

### Effects of Exercise Training on Peripheral Blood Cell Counts

Excessive training in athletes has been associated with increased susceptibility to infections, as a result of chronic immunosuppression.<sup>35</sup> Furthermore, acute exercise is known to induce peripheral leukocytosis.<sup>36</sup> This leukocyte response is biphasic: after an immediate transient response of lymphocytosis, monocytosis, and neutrophilia, a delayed response comprising mainly neutrophilia occurs. Longer term exhaustive endurance exercise such as a marathon or triathlon is predominantly associated with this delayed neutrophilia.<sup>37,38</sup> The proposed mechanism is an increase in the stress hormone cortisol above 60% of maximum oxygen uptake (VO<sub>2</sub> max).<sup>38</sup> A longitudinal study by Suzuki et al<sup>39</sup> addressed the adaptability of these blood cell responses to training. Ten healthy untrained men performed exercise sessions (at an intensity of 70% of VO<sub>2</sub> max) for 1.5 hour each day, for 7 consecutive days. Whereas the first training session caused marked peripheral neutrophilia, in particular band neutrophils, the magnitude of the exercise-induced changes reduced gradually (although not significantly) by daily repeated exposure to endurance exercise. However, none of the trends were significant except the decline in resting segmented neutrophil counts.<sup>39</sup> In our cycling study (see the ►Appendix), we observed a similar cellular response to repeated exercise (►Fig. 1), characterized by an increase in granulocytes and decrease in monocytes and lymphocytes on the first day after exercise, followed by



**Fig. 1** Exercise-induced lymphocytosis, monocytosis, and neutrophilia. On the first day, the number of granulocytes was significantly increased after cycling. In contrast, monocyte and lymphocyte levels decreased each day after cycling. On the second and third day, levels returned to baseline. Platelets, red blood cell count, hematocrit, and hemoglobin remained largely stable. Data represent the mean ± standard deviation (SD) (n = 5).

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recovery to baseline each day before exercise. On the first day, the number of granulocytes was significantly increased after cycling. On the second and third day, this level returned to baseline, but cycling evoked a smaller increase in granulocyte levels compared with the first day. In contrast, monocyte and lymphocyte levels decreased significantly on day 1 each day after cycling, but also returned to baseline each day before the cycling. Platelets, red blood cell (RBC) count, hematocrit, and hemoglobin remained largely stable.

## Effects of Exercise Training on Hemostasis

► **Table 1** provides an overview of studies investigating the effect of training on a variety of hemostatic parameters.

**Table 1** Overview of studies and reported (direction of the) effect of training (physically trained compared with sedentary individuals or post- versus pretraining program in the same individual) on hemostatic parameters

	Parameter		Effect	Reference
Coagulation	Clotting times	aPTT	↓	Korsan-Bengtzen et al <sup>53</sup>
		aPTT	↑	Hilberg et al <sup>54</sup> ; Kupchak et al <sup>55</sup>
		aPTT	=	Ferguson et al <sup>50</sup>
		PT	=	Ferguson et al <sup>50</sup> ; El-Sayed et al <sup>52</sup> ; Korsan-Bengtzen et al <sup>53</sup>
		TT	=	Watts <sup>51</sup> ; El-Sayed et al <sup>52</sup>
	FVIII	Ag	↑	Lockard et al <sup>79</sup>
			=	Boman et al <sup>76</sup> ; Watts <sup>51</sup> ; Rankinen et al <sup>77</sup> ; Ponjee et al <sup>45</sup> ; van den Burg et al <sup>78</sup> ; Lippi et al <sup>64</sup> ; Korsan-Bengtzen et al <sup>53</sup>
	VWF	Ag/Act	↓	Wang et al <sup>44</sup>
	Thrombin	ETP	↓	Cimenti et al <sup>131</sup>
			↓	Hilberg et al <sup>54</sup> ; Lockard et al <sup>79</sup> ; Kupchak et al <sup>55</sup>
			=	Kupchak et al <sup>55</sup>
	Fibrinogen	Ag	↓	El-Sayed <sup>90</sup> ; Stratton et al <sup>85</sup> (elderly men)
			↑	Schuit et al <sup>92</sup> (elderly men)
=			Stratton et al <sup>85</sup> (young men); DeSouza et al <sup>93</sup> (elderly women); Kupchak et al <sup>55</sup>	
Platelets	Aggregation Activation		↓	Wang et al <sup>44</sup>
		P-sel	↓	Santilli et al <sup>108</sup> ; Wang et al <sup>44</sup>
		VWF bind.	↓	Wang et al <sup>44</sup>
		PF4	↑	Ponjee et al <sup>45</sup>
			=	Creighton et al <sup>47</sup>
		βTG	↓	Davis et al <sup>42</sup> ; Gonzales et al <sup>46</sup> ; Creighton et al <sup>47</sup>
GPIb/GPIV	↓	Kestin et al <sup>43</sup>		
Fibrinolysis	Clot lysis time		=	Ferguson et al <sup>50</sup> ; Korsan-Bengtzen et al <sup>53</sup>
	tPA	Ag/Act	↑	Szymanski et al <sup>84</sup> ; De Paz et al <sup>100</sup> ; Kupchak et al <sup>55</sup> ;
	PAI-1	Ag	↓	Kulaputana et al <sup>125</sup>
			↑	De Geus et al <sup>101</sup> ; Gris et al <sup>102</sup> ; Kupchak et al <sup>55</sup> ;
		=	Kulaputana et al <sup>125</sup> ; Speiser et al <sup>103</sup> ; Estellés et al <sup>104</sup>	

Abbreviations: =, no change, in response to physical training/increased training status; ↑, increase; ↓, decrease; Act, activity; Ag, antigen; aPTT, activated partial thromboplastin time; βTG, β thromboglobulin; ETP, endogenous thrombin potential; F1 + 2, prothrombin fragment 1 and 2; GPIb/GPIV, glycoprotein Ib/glycoprotein IV; PAI-1, plasminogen activator inhibitor-1; PF4, platelet factor 4; P-sel, P-selectin; PT, prothrombin time; TAT, thrombin-antithrombin complex; tPA, tissue plasminogen activator; TT, thrombin time; VWF bind, von Willebrand factor binding.

## Primary Hemostasis

### Platelet Activity

Platelet reactivity has a stable association with the occurrence of acute thrombosis and the long-term pathogenesis of thrombosis and cardiovascular diseases.<sup>40,41</sup> However, the effects of physical training on platelet function have not been fully discerned. It is hypothesized that training in healthy individuals could reduce the risk of cardiovascular disease by suppressing platelet reactivity. Supporting this hypothesis is the observed decrease in exercise-induced platelet hyperreactivity as a result of endurance training in previously sedentary individuals.<sup>42–44</sup>

Two studies investigated the effect of training on platelet activation markers platelet factor 4 (PF4) and β-thromboglobulin

( $\beta$ TG). In a prospective study, a 9-month endurance training program resulted in slightly increased PF4, but no significant change in  $\beta$ TG levels in men and women, suggesting unfavorable training-induced *in vivo* platelet activation.<sup>45</sup> In contrast, resting  $\beta$ TG levels in physically fit individuals have been reported to be lower compared with sedentary controls.<sup>42,46</sup> Thus, an improved training status appears to have different effects on resting platelet activation than a long-term training program, but further studies in larger populations are warranted to investigate this hypothesis and the underlying mechanism.

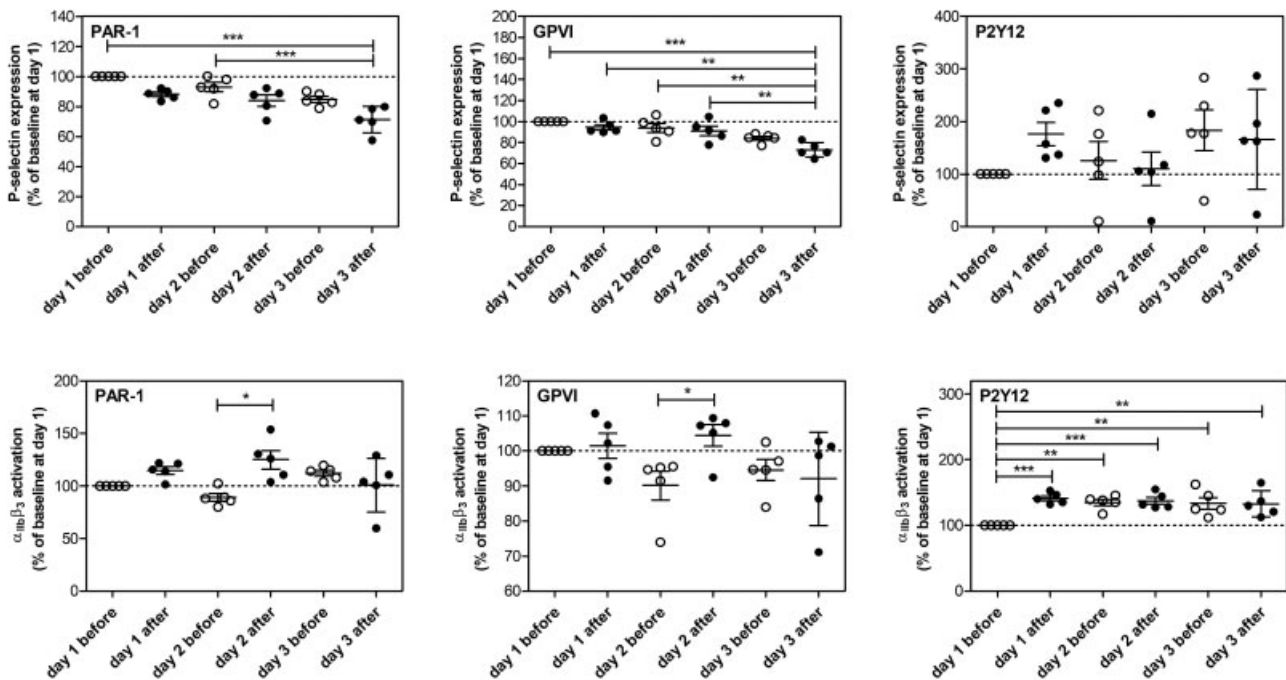
In a study by Creighton et al, the hemostatic recovery after exercise in terms of platelet  $\beta$ TG and PF4 was monitored with enzyme-linked immunosorbent assays (ELISAs) instead of radioimmunoassays. In the first hour postexercise, resistance-trained individuals displayed significantly reduced platelet activation, in terms of lower  $\beta$ TG levels, compared with untrained individuals.<sup>47</sup> Of note, PF4 and  $\beta$ TG radioimmunoassays and ELISAs are associated with some methodological difficulties.<sup>43,48</sup> An alternative analysis method for platelet activation is whole blood flow cytometry using antibodies against platelet activation markers, for example, glycoprotein (GP) Ib and GPIV. Using this assay, strenuous exercise was found to induce both platelet activation and platelet hyperreactivity in sedentary subjects but not in physically trained subjects.<sup>43</sup>

In our cycling study, platelet activation was also measured in whole blood with a flow-cytometric platelet function test ( $\rightarrow$  Fig. 2).<sup>49</sup> Granule release potential was measured as P-selectin expression and the aggregation potential was measured as activation of the  $\alpha$ IIb $\beta$ 3 receptor, both after adding the platelet agonists thrombin receptor-activating peptide (TRAP), collagen-related peptide (CRP), or 2-methylthio-adenosine diphosphate (Mes-ADP). Postcycling, P-selectin expression

showed a similar decreasing pattern for protease-activated receptor-1 (PAR-1) stimulation by TRAP and GPIV stimulation by CRP. Interestingly, there was no recovery to baseline the next day before cycling, and P-selectin expression in response to these agonists dropped significantly on the third day. In contrast,  $\alpha$ IIb $\beta$ 3 expression increased slightly after exercise but decreased to baseline for PAR-1, or even lower than baseline for GPIV, on the next day prior to exercise. Mes-ADP-mediated P2Y12 stimulation induced increased P-selectin expression and  $\alpha$ IIb $\beta$ 3 activation the first day after exercise, but remained rather stable on the subsequent days. In summary, repeated exercise predominantly induced an exhaustive effect on platelet granule secretion (P-selectin expression) in response to TRAP and CRP.

### Clotting Times

The conventional prothrombin time (PT) and aPTT are measures of overall plasma coagulation via different pathways, and measure the time needed for clot formation; a shortened clotting time indicates a prothrombotic state, whereas increased clotting time indicates hypocoagulability. The PT and aPTT were not different between joggers, marathon runners, and individuals with a sedentary lifestyle, both at rest and after exercise.<sup>50</sup> These findings are in accordance with a later study reporting a similar thrombin time (TT) at rest in athletes and nonathletic individuals.<sup>51</sup> Moreover, one of the few longitudinal studies investigating the long-term effects of training on coagulation demonstrated no significant change in TT and PT after 3 months of endurance training.<sup>52</sup> However, although it can be debated whether daily physical activity can be considered training, a large cohort study including 772 men (all aged 54 years) found



**Fig. 2** Repetitive exercise induces exhaustion of the P-selectin expression in response to protease-activated receptor (PAR)-1 and glycoprotein (GP) IV agonists. Data are presented as mean  $\pm$  standard deviation (SD) ( $n = 5$ ).

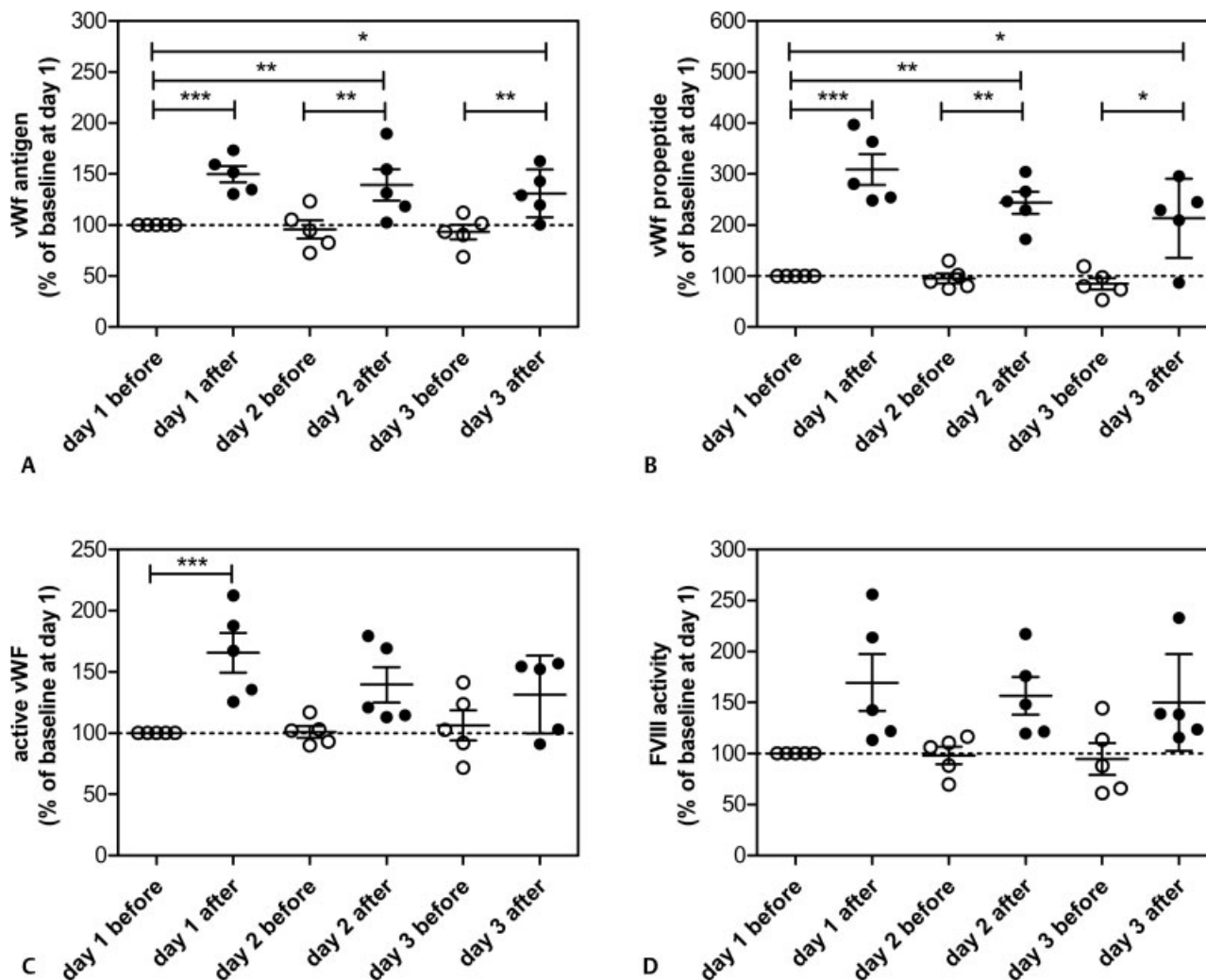
that physically very active individuals (assessed by a questionnaire) did have a significantly lower aPTT, indicative of a hypercoagulable state, compared with nonactive individuals.<sup>53</sup> In contrast, one of the few randomized controlled studies found a small but significant prolongation of the aPTT, meaning a more hypocoagulable state, at rest and postexercise after 12 weeks of moderate aerobic endurance training.<sup>54</sup> The latter was confirmed by a more recent study in which trained subjects had a lower clot-forming capacity (prolonged aPTT) following an acute exhaustive resistance exercise test than untrained subjects.<sup>55</sup> Taken together, data on the effects of training on clotting times are inconclusive. Of note, both PT and aPTT are relatively “crude” screening tests that may not be sensitive enough to detect hypercoagulability. Therefore, further studies should focus on more sensitive biomarkers of coagulation.

### von Willebrand Factor

von Willebrand factor is a large multimeric plasma GP with essential functions in hemostasis.<sup>56</sup> Several agonists, including

hypoxia, epinephrine, histamine, thrombin, fibrin, and vasopressin, trigger release of hyperreactive ultra-large VWF multimers from endothelial cell Weibel–Palade bodies.<sup>57,58</sup> Importantly, this endothelial cell activation also occurs after both short-duration exhaustive exercise<sup>30</sup> and long-duration vigorous exercise,<sup>16–18</sup> resulting in an increase in circulating VWF levels.

In our cycling study in five healthy and physically fit individuals, all VWF parameters (VWF antigen [VWF:Ag], VWF propeptide [VWF:pp], and active VWF) were significantly increased on the first day after cycling (► Fig. 3A–C). It is well known that levels of VWF:Ag increase steeply upon acute intense physical exercise.<sup>59</sup> Also, several studies demonstrated exercise-induced elevation of VWF activity, as measured by collagen-binding assays<sup>60</sup> or ristocetin cofactor assays.<sup>61</sup> However, not many studies have determined VWF:pp and active VWF (see the ►Appendix) levels in response to exercise. VWF:pp is critical for intracellular processing of VWF by endothelial cells. It was previously established that by assaying both VWF:pp and VWF:Ag, one



**Fig. 3** Repetitive exercise induces an endothelial adaptive response. The magnitude of the exercise-induced increase in (A) von Willebrand factor (VWF) antigen (VWF:Ag), (B) VWF propeptide (VWF:pp), (C) active VWF, and (D) factor VIII (FVIII) is less every subsequent day after cycling. (D) FVIII activity was measured as described previously.<sup>126,127</sup> All measurements were performed in triplicate, and data represent the mean  $\pm$  standard deviation (SD) ( $n = 5$ ).

can distinguish between acute and chronic endothelial cell damage.<sup>48</sup> In healthy controls, a concomitant rise of VWF:pp and VWF:Ag levels, for example, after exercise-induced release from endothelial cell storage, is followed by a rapid decline in VWF:pp level, whereas VWF:Ag levels return to baseline more slowly.<sup>62</sup> Active VWF level increases as a result of strenuous exercise due to high intravascular shear stress, which induces VWF to unfold and expose the A1 domain; this conformation is known as active VWF. The observed post-exercise increase in active VWF is in accordance with those reported for the healthy control group in a study on the effects of exercise on VWF in type 1 and type 2B von Willibrand disease patients.<sup>61</sup> In our cycling study, on the second and third day before cycling all VWF parameters had returned to baseline. Strikingly, on these days all VWF parameters on average increased slightly less in response to cycling compared with the effect on the first day, suggesting that already within 3 days either exhaustion of the endothelial response to physical stress or exhaustion of the VWF supply, similar to tachyphylaxis after repeated DDAVP administration, can be observed. In line with this, the endothelium is known for being modifiable depending on its environment, by mediating pro- and anticoagulant systems. In fact, a previous study demonstrated that nitric oxide (NO)-dependent endothelial functions can adapt favorably in as little as 1 week of endurance training.<sup>63</sup>

Conflicting data are reported on the effect of training status on VWF levels. While VWF:Ag levels did not differ between professional cyclists and sedentary individuals,<sup>64</sup> the increase in VWF:Ag upon strenuous exercise appears strongly dependent on performance (among others peak power output/kg body weight and VO<sub>2</sub> peak/kg) and physical fitness-related determinants (among others VO<sub>2</sub> at ventilatory threshold and power output at ventilatory threshold). Interestingly, this increase in VWF:Ag level upon strenuous exercise was highest in individuals who are the least physically fit but also in those who regularly performed very high-intensity exercise.<sup>60</sup> In a cohort of equally physically fit subjects, an 8-week training program reduced levels of resting and postexercise VWF:Ag and activity. However, deconditioning completely reverses this training effect.<sup>65</sup>

From a mechanistic point of view, VWF:Ag stored in Weibel-Palade bodies of endothelial cells and platelets may be released more rapidly: (1) as a result of training-induced upregulation of  $\beta$ 2-adrenoreceptors,<sup>66</sup> and (2) to compensate for shear stress induced by agonists such as thrombin, collagen,<sup>67</sup> epinephrine,<sup>68</sup> and vasopressin.<sup>69</sup> For instance, vasopressin increases blood pressure and vascular resistance during exercise, thereby increasing shear stress. Strenuous physical exercise also results in recruitment of capillaries in muscles and an increase in vascular conductance in these muscles.<sup>70</sup> As a consequence, the endothelium of trained individuals is exposed to more shear stress and more adrenergic stimulation, which may explain the increased release of VWF upon exercise.<sup>71</sup> Of note, shear stress is also known to unfold VWF multimers, exposing the A1 domain.<sup>72</sup> This active conformation of VWF can more readily bind platelets and is thus more thrombogenic.<sup>73,74</sup>

In conclusion, exposure to exercise in trained individuals may facilitate a more rapid return of hemostatic parameters such as VWF to resting conditions. Hence, if this hypothesis would hold true, the release of VWF:Ag may be briefer in trained subjects.<sup>75</sup>

## Secondary Hemostasis

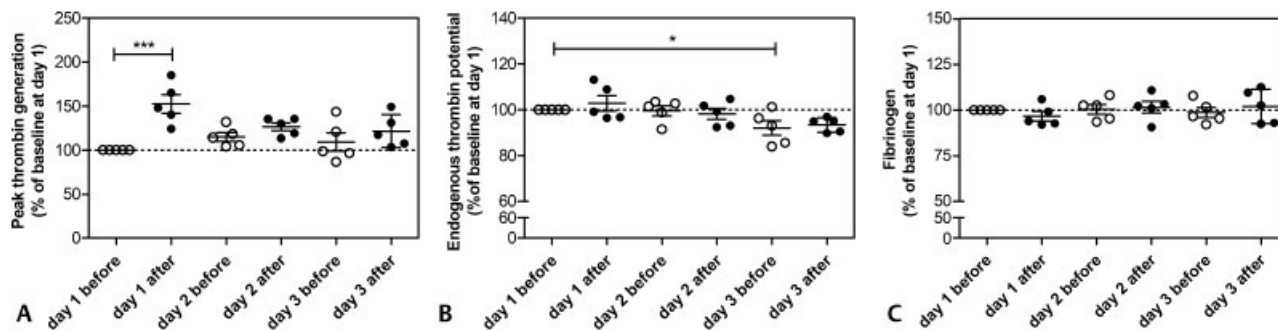
### Factor VIII

Resting levels of FVIII activity and FVIII antigen do not change with training in physically fit,<sup>51</sup> as well as sedentary individuals in the majority of studies.<sup>45,52,64,76–78</sup> In a more recent study, FVIII:Ag levels did increase significantly with training,<sup>79</sup> consistent with the observed increase in FVIII activity after acute exercise.<sup>25,30,80,81</sup> However, this inconsistency may be explained by population differences, as the latter study included elderly men and women (50–75 years), whereas all other studies mentioned previously were performed with young individuals. In our cycling study, the submaximal intensity cycling induced an increase in FVIII activity on each of three subsequent days (–Fig. 3D). Interestingly, the difference between pre- and postexercise FVIII levels became smaller every day, indicating either adaptation of the FVIII response to exercise or exhaustion of the FVIII supplies. FVIII circulates in complex with VWF, therefore high FVIII levels correlate with an increased level of VWF. Of note, the aforementioned shortening of the aPTT and the elevation in FVIII activity induced by acute strenuous exercise are both indicators for contact factor-mediated clotting activity. Hence, this pathway may have an important role in postexercise hypercoagulability,<sup>82</sup> but does not appear subject to an adaptive response induced by training.

### Thrombin

In contrast to global clotting assays such as the PT and aPTT, measurement of TG allows assessment of the full process of coagulation over time, providing greater sensitivity.<sup>83</sup> Thrombin formation is lower in trained versus sedentary subjects as shown by a lower resting endogenous thrombin potential (ETP)<sup>84</sup> and lower level of prothrombin fragments F1 + 2, a surrogate thrombin marker.<sup>79</sup> In addition, in endurance-trained subjects the increase of thrombin formation in response to exercise is less pronounced compared with controls.<sup>85</sup>

Regarding the short-term effect of repetitive exercise on TG, we observed in our cycling study that peak TG was significantly increased on the first day following exercise (–Fig. 4A). However, peak TG returned back to baseline before cycling on the second and third day. This is in accordance with findings from a recent study in which an acute bout of high-intensity exercise increased TG immediately after exercise, but returned to baseline the following day.<sup>20</sup> Remarkably, almost no increase in ETP was observed after cycling on the second and third day. The ETP before exercise actually decreased over the 3 days of cycling (–Fig. 4B). Together, these results show an initial surge, followed by exhaustion of the thrombin-generating capacity of the hemostatic system. This pattern possibly reflects the



**Fig. 4** Repetitive exercise induces an adaptive response of the thrombin generation peak (A) and exhaustion of the endogenous thrombin potential (ETP) (B), but does not affect fibrinogen levels (C). Thrombin generation parameters recorded were peak thrombin (panel A) and ETP (panel B). Data are presented as the mean  $\pm$  standard deviation (SD) ( $n = 5$ ).

observed exercise-induced increase in FVIII with a tapered effect over the 3 days. Our study only lasted 3 days, but these results warrant further investigations to determine whether the drop in TG continues further and how much time after exhaustion is required for TG to return to baseline.

Overall, the apparent favorable effect of training on clotting potential may explain the lower incidence of thrombosis in physically fit individuals, despite the transiently hypercoagulable state during and directly after strenuous exercise.

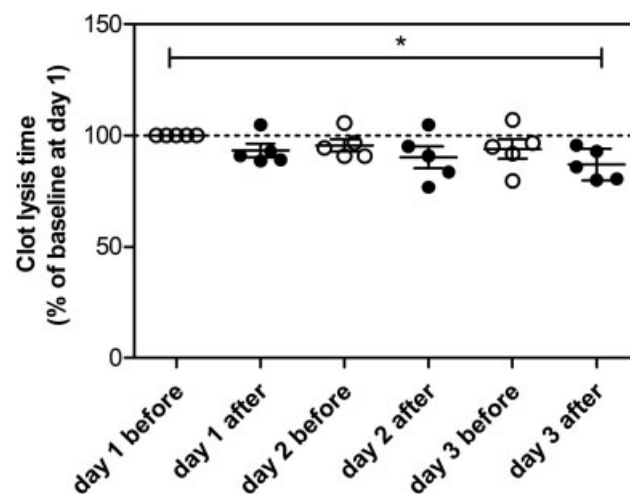
### Fibrinogen

Fibrinogen plays a pivotal role in normal hemostasis, representing the substrate for conversion to fibrin and supporting TG and platelet aggregation.<sup>86</sup> Studies on the effects of exercise on fibrinogen levels have produced conflicting data, reporting significant increases, decreases, or no effects.<sup>30,80,84,87–89</sup> These inconsistencies in the literature can likely be attributed to differences in experimental design, including duration, mode and intensity of exercise, health and training status of the study population, and analytical methods.<sup>30,80,87</sup> Whereas most studies determined the effects of acute exercise, the influence of physical training on plasma fibrinogen levels is less well studied. Cross-sectional studies suggest a favorable effect of regular physical activity, decreasing plasma fibrinogen levels.<sup>90,91</sup> However, longitudinal data are sparse and conflicting. Physical training at moderate intensity reduced plasma fibrinogen levels in elderly men but not in young men.<sup>85</sup> In contrast, following intensive training plasma fibrinogen levels were increased in elderly men, paralleled by a surge in C-reactive protein suggesting a chronic increase in acute-phase reactant proteins.<sup>92</sup> In elderly women, on the other hand, no significant effects of training on fibrinogen levels were found.<sup>93</sup> Similarly, in our cycling study in men, no significant changes in fibrinogen plasma levels in response to repeated bouts of submaximal exercise were observed ( $\rightarrow$  Fig. 4C). Altogether, there is a lack of conclusive evidence on the exact effects of physical training on plasma fibrinogen levels.

### Fibrinolysis

In spite of the hypercoagulable state that occurs during and directly after strenuous exercise, regular physical activity has been associated with a reduction in cardiovascular

risk.<sup>94–96</sup> The underlying mechanisms of this conditioning effect remain speculative, but are often ascribed to favorable effects on fibrinolysis.<sup>52,97–99</sup> However, as with the effects on coagulation, studies on the effects of training on fibrinolysis parameters have produced inconsistent results. Global assays of fibrinolysis, for example, the clot lysis time, found no difference in fibrinolytic capacity between marathon runners and less active individuals.<sup>50,53</sup> In our small-scale cycling study, clot lysis time decreased only very slightly on the first 2 days after exercise and decreased significantly on the third day ( $\rightarrow$  Fig. 5). When assaying specific fibrinolytic factors, effects of exercise could be observed. For instance, inactive individuals have higher tissue plasminogen activator (tPA) activity and tPA antigen levels compared with active individuals.<sup>84,100</sup> Data on plasminogen activator inhibitor (PAI)-1 levels following training are inconclusive. A favorable reduction in PAI-1 activity was observed after 8 months of training (albeit not significant due to large group variances and seasonal variations)<sup>101</sup> and after 3 months of training.<sup>102</sup> Detraining for 3 months in the latter study reversed the reduction in PAI-1 activity.<sup>102</sup> On the other hand, PAI-1 values were increased in athletes compared with age-



**Fig. 5** Repetitive exercise causes only a minor decrease of clot lysis time (CLT) over the course of 3 days. The CLT (50% fibrinolysis) is defined as the time between maximal and half-maximal turbidity. Data are presented as mean  $\pm$  standard deviation (SD) ( $n = 5$ ).

matched sedentary individuals and elderly sportsmen<sup>103</sup> and an exercise rehabilitation program did not significantly reduce PAI-1 levels in healthy controls.<sup>104</sup>

Several mechanisms may be responsible for training-induced adaptation of fibrinolysis. One explanation is the enhanced sensitivity of the endothelium to release tPA, possibly paralleled by reduced clearance of tPA by the liver. In addition, formation of tPA/PAI-1 complexes is reduced,<sup>100,105</sup> which may be related to exercise-induced changes in lipid profile that lower resting PAI-1 activity.<sup>80,106,107</sup> Catecholamines may also play a role in training-related changes in fibrinolysis through their effects on the endothelium. However, data on the magnitude of the effect of plasma epinephrine and norepinephrine are equivocal, and hence the enhanced sensitivity of the endothelium to release tPA may be related to other, nonadrenergic mechanisms.

In conclusion, although the available evidence is derived from highly heterogeneous study protocols, training may have a positive influence on fibrinolysis in terms of an increase in tPA levels and a reduction in PAI-1 levels, although this must be verified in future studies before a definitive conclusion can be drawn.

## Modifiers of the Effects of Training on Hemostasis

### Lipid Profile and Nitric Oxide

Changes in lipid profile and plasma NO levels (as measured by NO metabolites) may modulate platelet reactivity in trained subjects.<sup>44</sup> Intensive repeated exercise resulted in significantly decreased thromboxane (TX)-dependent platelet activation (urinary excretion of 11-dehydroTXB<sub>2</sub>, enzymatic metabolite of TXA<sub>2</sub>), TX-independent platelet activation (plasma P-selectin), and platelet-derived inflammatory proteins (plasma CD40L) in otherwise sedentary subjects. Concomitantly, a significant increase in high-density lipoprotein (HDL) cholesterol concentration, linearly and inversely related to changes in the P-selectin and CD40L platelet activation markers, was observed.<sup>108</sup> Indeed, HDL is known to engage in a large number of beneficial activities at the endothelial level, among others stimulating NO synthase and inhibiting platelet activation and aggregation,<sup>109</sup> which may explain mechanistically how this type of high-intensity regular physical training may reduce the risk of cardiovascular events.<sup>110</sup>

### Catecholamines

Regular exercise and training decreases surges in catecholamines, both at rest and following exercise.<sup>26</sup> Only 1 week of vigorous exercise training was sufficient to induce significant reductions in the norepinephrine response to the same workload.<sup>111</sup> However, catecholamines have distinct effects on different key players in blood coagulation. For example, training reduces the density and affinity of platelet surface adrenergic receptors. Together with a decreased release of catecholamines in response to exercise, this leads to reduced overall platelet activity and aggregation under high shear flow.<sup>112,113</sup> In contrast, training-induced upregulation of endothelial  $\beta_2$ -adrenoreceptors causes accelerated release

of FVIII and VWF,<sup>66</sup> which may result in a more procoagulant phenotype after training.

### Dehydration

During exercise, sweating and intravascular fluid shifts into the interstitium resulting in decreased plasma volume without a proportional loss of plasma proteins.<sup>114</sup> This hemoconcentration may result in increased postexercise (coagulation) protein levels (falsely suggesting increased activation of the coagulation system) and changes in RBC count, hemoglobin, and hematocrit.<sup>114</sup> However, whereas in most studies on the effects of short-term exercise plasma volume was decreased after exercise, coagulation factor levels did not change.<sup>115–117</sup> In one report, an increased hematocrit was accompanied by increased levels of FIX, FXI, and FXII, but no changes in other coagulation factors were observed, suggesting actual activation of the contact pathway of coagulation.<sup>25</sup> In our cycling study, no changes in hematocrit and hemoglobin and a minor decrease in RBC count were observed. Hence, hemoconcentration is not expected to distort our findings of increased FVIII levels, TG, and VWF following exercise.

### Training Status and Mode

One factor that modifies the effect of physical activity on fibrinolysis is the training status of the individual.<sup>50</sup> The activating effects of exercise on hemostasis appear more pronounced in sedentary compared with physically fit individuals.<sup>59</sup> For instance, strenuous exercise caused significant platelet hyperactivation in sedentary men, but not in regularly exercising subjects.<sup>43,111</sup> Likewise, posttraining tPA release was increased and tPA/PAI-1 complex decreased in physically trained subjects compared with untrained individuals.<sup>84,100</sup> Training status influences an individual's maximal aerobic capacity, and studies have found that the level of acceleration of fibrinolytic activity is directly related to the workload, suggesting that higher aerobic fitness may lead to a larger increase in fibrinolysis with maximal exercise.<sup>50,80</sup> Exercise mode may also influence the hemostatic response to training. Above-mentioned studies show that endurance training seems to condition platelets to become less activated and aggregate less in response to strenuous exercise.<sup>43,44</sup> In contrast, resistance training is believed to enhance hemostatic functions by increasing vessel diameter and promoting antithrombotic endothelial activity.<sup>118–121</sup>

### Age

The favorable effects of training on blood fibrinolysis may be at least partly dependent on age. In the elderly, there is a higher fibrinolytic capacity, characterized by increased tPA and decreased PAI-1 activity<sup>85</sup> and antigen<sup>92</sup> after training, compared with young individuals. However, another study reported that physical training can also positively influence blood fibrinolysis in younger individuals.<sup>78</sup> In addition, plasma fibrinogen levels reduced in response to training in older individuals, but not in young subjects.<sup>59,85</sup> Other studies have found improvements in hemostatic markers, including PT, FVIII, prothrombin F1 + 2, and VWF in subjects between 50 and 75 years old but not in a younger population.<sup>122,123</sup>



## Gender

Most studies examining the hemostatic effects of training included only male subjects. The response of peripheral blood cell counts and D-dimer levels to a single bout of exercise appear the same in both genders, whereas the postexercise clotting times decreased more in males than females.<sup>124</sup> The sparse data available for repetitive exercise suggests that gender may modulate the fibrinolytic response to training, as endurance training-induced improvements in endogenous fibrinolysis markers, that is, a decrease in PAI-1 and increase in tPA levels, are somewhat greater in men compared with women.<sup>125</sup>

## Conclusion

Although exercise is established to be essential for a healthy lifestyle, there are many reports of exercise-induced thrombotic events. This so-called exercise paradox<sup>11,12</sup> reflects the complex interplay between hemostasis and physical stress. Whereas the risk of a cardiovascular event after vigorous exercise is increased in untrained individuals, training might induce adaptations of the hemostatic system, explaining its favorable effects on cardiovascular health and mortality.

Primary and secondary hemostasis, as well as fibrinolysis are all affected by training. However, the exact effects of exercise and training on the hemostatic profile and the underlying mechanisms remain to be elucidated. Inconsistencies in reported data, as apparent from ► **Table 1**, can be attributed to variation in subject-related factors such as age, gender, diet, and training status, as well as activity-related factors such as intensity, duration, and mode of exercise. While the latter factors can be standardized by investigators, study populations are generally heterogeneous, precluding definite conclusions on the size and direction of the effects of repeated exercise on pro- and anticoagulant processes.

Furthermore, almost no data on the short-term effects of repeated exercise are reported in the literature. For this reason, we performed a pilot study in which five individuals cycled at submaximal intensity for three subsequent days. The results of this pilot study suggest that repeated submaximal exercise leads to exhaustion of several components of the hemostatic system, predominantly of the endothelium (apparent from changes in VWF and FVIII) and platelet granule secretion (as measured by P-selectin expression).

We acknowledge several limitations of our pilot study that could have influenced the measured hemostatic parameters. First of all, the limited reproducibility of our “exercise protocol,” that is, cycling a specific route in the hilly landscape in the south of the Netherlands, and the inability to monitor and control exercise intensity or environmental conditions are important weaknesses of this study. Therefore, in further studies it is recommended to perform a standardized submaximal exercise protocol on a cycling ergometer. In addition, participants were considered physically fit based on their self-reported frequency and intensity of exercise, but this was not objectively determined (e.g., in terms of aerobic capacity  $V_{O2}$  max) prior to the pilot study. Thus, observed differences in hemostatic response to bouts of strenuous exercise may be

partially accounted for by differences in baseline fitness. Finally, participants consumed water, bananas, energy drinks, energy bars, and energy gels ad libitum during cycling, but the individual fluid and food intake was not recorded.

Several questions related to the effects of repetitive exercise and hemostasis remain unanswered and warrant further investigation. For instance, there is a large gap in knowledge on the effects of training on a crucial factor in fibrinolysis, namely, plasminogen levels and subsequent plasmin generation. Moreover, it is highly relevant to obtain better estimates of the impact of training status on the incidence of exercise-induced thrombotic events, and how this compares to the effects of therapeutic intervention. Ideally, these studies should include a longitudinal design with a follow-up of several years. In addition, new research should focus on biomarkers of training-induced endothelial activation, in particular (active) VWF. Finally, more studies on short-term repetitive exercise on hemostasis are required, as the effects on hemostasis appear different than those induced by long-term repetitive exercise in the form of training. Altogether, insights into the effects of training on hemostatic disturbances by exercise will form the basis to make the double-edged sword of exercise cut in the favorable direction, protecting against thromboembolic morbidity and mortality.

## Conflicts of Interest

None.

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## Appendix: Methodology for the Pilot Study

### Study Population

Five physically fit (nonprofessional) male cyclists were included in the pilot study and cycled 80 km on each of three consecutive days. All participants were nonsmokers, none had a known hemostatic or cardiovascular disease, and none used antiplatelet/anticoagulant drugs for 1 week prior to the study. All participants gave written informed consent before the study and blood withdrawal was approved by the medical research ethics committee of Maastricht University Medical Centre.

### Repetitive Exercise

On each of three subsequent days, the participants cycled approximately 80 km over the course of 4 hours, always at the same time of day to take into account possible diurnal effects.<sup>24</sup> Submaximal exercise intensity was achieved by cycling in a hilly landscape, covering a total of 800 height meters, with a maximum slope of 10%, inducing 90 to 95% intensity for approximately 10 minutes when climbing hills, and > 75% intensity between hills.

### Blood Sample Collection

Blood samples were collected before and immediately after exercise on each of the three study days. Venous blood was collected in 3.2% (w/v) citrated Vacutainer tubes (Becton Dickinson (BD) Vacutainer System).

### Analytical Methods

To characterize changes in their hemostatic profile, we measured VWF (total antigen,<sup>126</sup> VWF propeptide,<sup>62</sup> and active VWF) levels, FVIII,<sup>126,127</sup> thrombin generation,<sup>128</sup> fibrinogen levels,<sup>127</sup> platelet activation,<sup>129</sup> and clot lysis time<sup>127</sup> (methods according to references). Peripheral blood cell counts in citrated whole blood were determined using a

COULTER counter analyzer (Beckman Coulter, Woerden, the Netherlands). Since the active VWF and VWF:Ag assays as used in our laboratory have not been published before, they are described in detail below.

### Active VWF and VWF:Ag Assays

VWF:Ag and active VWF were measured in plasma by enzyme-linked immunosorbent assays (ELISAs). The active VWF assay is based on a llama-derived variable heavy chain (VHH) directed against the A1 domain of VWF, which is only exposed upon unfolding of VWF.<sup>130</sup> Briefly, 96 wells microtiter plates (NUNC Maxisorp, Thermo Fisher Scientific, USA) were coated overnight at 4°C with VHH against active VWF or with polyclonal antibody against human VWF (A0082, Dako, Denmark) (VWF:Ag) and blocked with 2% bovine serum albumin (BSA; Sigma, USA) in phosphate-buffered saline (PBS) for 45 minutes at room temperature. After extensive washing with 0.01% Tween-20 in PBS, plasma samples (diluted in PBS/1% BSA) were incubated for 2 hours at room temperature. Following another round of washing, the wells were incubated with horseradish peroxidase (HRP)-conjugated polyclonal anti-VWF (P0226, Dako, Denmark) in PBS/1% BSA for 2 hours at room temperature. Plates were then washed three times more before measuring the binding of active VWF to the VHH or VWF:Ag to the polyclonal anti-VWF antibodies by using SIGMAFAST OPD (Sigma, USA) as a substrate for HRP. The substrate reaction was stopped with 3 M sulfuric acid (H<sub>2</sub>SO<sub>4</sub>, Aldrich, USA). Optical densities (ODs) were measured at 490 nm using an ELx808 Absorbance Microplate Reader (Biotek, USA). Normal pooled plasma (NPP) was used as a standard in every plate and plasma sample results were normalized (%) to NPP on the same plate. The results of these analyses are described below each corresponding figure, as referred to in the main text of this review.