# Fainting induces an acute increase in the concentration of plasma factor VIII and von Willebrand factor

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*Background and Objectives.* von Willebrand factor (VWF) is stored in the Weibel-Palade bodies of endothelial cells and may be released in response to different secretion stimuli such as stress, physical exercise, adrenaline, DDAVP and thrombin.

*Design and Methods.* We found that fainting can also induce an acute increase in plasma VWF and factor FVIII (FVIII) concentrations, following observations in two patients with von Willebrand's disease (VWD) who experienced a fainting episode during venipuncture for blood collection.

*Results.* One patient was classified as having type Vicenza VWD, the other as type 1 VWD; both had normal platelet VWF content. After the fainting episode, FVIII and VWF levels were significantly higher than the levels in blood samples collected without stress; mean increases were 4.35-fold for FVIII, 4-fold for VWF:Ag and 5.3-fold for VWF:RCo, with values overshooting the upper limit of the normal range. Moreover, the post-fainting plasma VWF multimer pattern was characterized by a significant increase in all oligomers with the appearance of unusually large VWF multimers, similar to those observed following DDAVP infusion.

*Interpretation and Conclusions.* These findings demonstrate that fainting acts as a stimulus capable of inducing the release of VWF from endothelial cells, and further highlight the role of stress in determining hemostatic states potentially favorable to the development of thrombotic complications.

Key words: fainting, VWF, FVIII, VWF multimers, endothelial cells.

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ainting, a hemodynamic condition characterized by significant, acute hypotension, is apparently due to a failure of the vascular smooth muscle to constrict, as suggested by the marked increase in plasma catecholamine concentrations and activation of the renin–angiotensin system.[1-5] Endothelium plays a key role in guaranteeing systemic regulation of blood vessel tone by releasing catecholamines and vasopressin, and through local mechanisms. Endothelial cells are strategically located between circulating blood and vascular smooth muscle and have four main functions: control of coagulation, regulation of vascular tone, control of vascular permeability and regulation of leukocyte adhesion and trafficking. The endothelial cells' contribution to the control of smooth muscle tone is mediated by the secretion of substances that cause relaxation and contraction of the myocytes both under basal conditions and when activated by neurotransmitters, hormones or physical stimuli (shear stress). Endothelium-dependent vasodilatation occurs in response to acetylcholine, ATP, ADP, arachidonic acid, substance P, neurokinin A, 5-hydroxytryptamine, bradykinin, histamine, thrombin, etc.,[6-7] and is mediated by the release of nitric oxide (NO) and prostacyclin ($PGI_2$),[8,9] which are potent inhibitors of platelet aggregation.[10] Indeed, endothelial cells can produce a number of factors that have either a procoagulant or anticoagulant action. One of these is von Willebrand factor (VWF), a high molecular weight polymeric glycoprotein that has a fundamental role in platelet plug formation at the site of vascular injury.[11] Plasma VWF derives from the constitutive release of the VWF synthesized by endothelial cells, which in part is also stored in the Weibel–Palade bodies.[12] VWF is present in these organelles in its high molecular weight forms, which have greater hemostatic competence.[13] VWF may be released from the Weibel-Palade bodies in response to secretion stimuli such as thrombin, adrenaline, DDAVP, and physical exercise in order to guarantee the presence of hemostatically highly competent VWF in circulation.[14] A decrease or abnormality in VWF is responsible for von Willebrand's disease (VWD), the most common inherited bleeding disorder.[15] On the other hand, an increase in circulating VWF or the presence of higher than normal VWF multimers may be associated with thrombotic conditions. In this study we identified fainting as a new stimulus for the acute release of VWF from endothelial cells.

**Table 1. Main hemostatic findings in the two VWD patients studied.**

| Patients | B.T. (Min) | aPTT (sec) | RIPA (%) | FVIII (U/dL) | VWF:Ag (U/dL) | VWF:RCo (U/dL) | Plat.VWF:Ag (U/dL) | Mutations |
|---|---|---|---|---|---|---|---|---|
| P.P. | 2.10 | 41.5 | 51 | 35 | 20 | 15 | 89.6 | M740I+R1205H |
| C.L. | 2.15 | 39 | 76 | 50 | 42 | 34.7 | 67 | Unknown |
| Normal Range | <4 | 30-40 | 60-84 | 60-160 | 60-160 | 60-130 | 70-140 | |

## Design and Methods

Patients and normal volunteers were studied following their written informed consent, in accordance with the declaration of Helsinki. Blood samples were drawn from the antecubital vein and anticoagulated using 3.8% sodium citrate (1:10, v/v); samples for washed platelet preparations also contained 50 mM EDTA, 50 IU/mL Trasylol, 10 mM leupeptin and 60 mM NEM, as protease inhibitors.[16]

VWF ristocetin cofactor activity (VWF:RCo) was measured with normal washed, formalin-fixed platelets and 1 mg/mL ristocetin, as previously described.[17] VWF antigen (VWF:Ag) was determined by an ELISA method.[18] FVIII coagulant (FVIII:C) was measured by a one-stage method, using cephaloplastin as the activated cephalin. Platelets for VWF:Ag measurement were prepared by differential centrifugation in PBS buffer containing 3% EDTA, and resuspended at a final concentration of $10^6/\mu L$ cells, in PBS containing protease inhibitors.

VWF multimer analysis was performed on high-gelling temperature agarose (HGT-P) containing 0.1% sodium dodecyl sulphate (SDS), using 1.2% agarose to obtain a low-resolution analysis that revealed the presence of high molecular weight multimers. VWF multimers were detected by autoradiography after reaction with a purified anti-VWF $^{125}$I-antibody. Autoradiographs were analyzed by a densitometer scanner (LKB, Uppsala, Sweden). 1-desamino-8-d-arginine vasopressin (DDAVP) was given subcutaneously at a dose of 0.3 μg/Kg. Blood samples were collected before and 15, 30, 60, 120 and 180 min after DDAVP administration. To avoid fainting episodes, patients were allowed to lie down for 10 minutes before blood collection.

## Results

### Patients

Two VWD patients were studied; one patient (P.P.) was a 23-year old woman with a life-long history of mild bleeding. She was classified as having type Vicenza VWD and had the mutation G2470A, M740I in exon 17 and the mutation G3864A, R1205H in exon 27 of the VWF gene, which are the candidate mutations of type Vicenza VWD.[19,20] The same hemostatic defect was also present in her father. The second patient (C.L.) was a 33-year-old woman with a life-long history of bleeding. She was classified as having type 1 VWD, with a normal platelet VWF content; the genetic defect was transmitted as an autosomal dominant trait but no mutations in her VWF gene have been identified to date.

During venipuncture procedures, both patients experienced a fainting episode during which a significant drop in blood pressure was recorded (systolic pressure below 90 Hg mm). These episodes did not compromise the blood collection procedure; patient P.P. fainted during the collection of the pre-DDAVP blood sample, but the DDAVP test was completed. Moreover, in order to obtain satisfactory blood samples, in another circumstance the patients were allowed to lie down in a well-ventilated room for 10 minutes before venipuncture.

### Hemostatic results

Table 1 reports the hemostatic parameters determined in blood collected under non-stressful conditions (stress-free sample). Patient P.P. had significantly low levels of VWF:Ag and VWF:RCo and a less pronounced decrease in FVIII, while the platelet VWF:Ag value was within the normal range; the patient's plasma VWF multimer pattern was characterized by the presence of unusually large VWF forms, typical of type Vicenza VWD (Figure 1A), with a homogeneous decrease in all other oligomers present in normal subjects. In patient C.L. the levels of VWF:Ag and VWF:RCo were mildly decreased with a less pronounced decrease in FVIII and a normal platelet VWF:Ag; the patient's VWF multimer pattern showed a homogeneous decrease in all components, and no abnormally large forms (Figure 1B).

After fainting, there was a significant increase in the hemostatic parameters of both patients; aPTT was shortened (mean 34.2 sec vs mean 40.3 sec in the stress-free samples), and FVIII, VWF:Ag and VWF:RCo values increased significantly (Figure 2). The mean increases were 4.35-fold for FVIII, 4-fold for VWF:Ag and 5.3-fold for VWF:RCo. Platelet
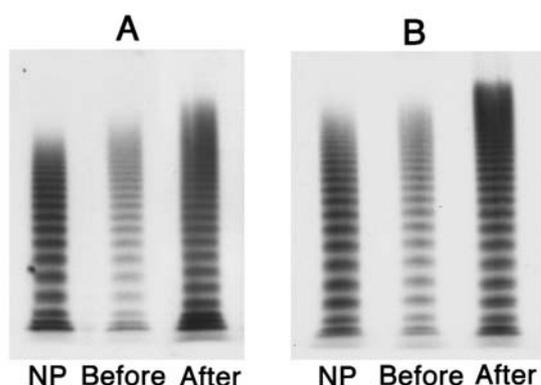
**Figure 1. Plasma VWF multimer pattern before and after fainting, in patients P.P. (A) and C.L. (B), and normal pooled plasma (NP). Multimer analysis was performed with 1.2% agarose gel electrophoresis, under non-reducing conditions. After the run, multimers were detected by autoradiography, using a ^{125}I-anti-VWF antibody. High molecular weight forms are at the top, low molecular weight forms at the bottom. Patient C.L. shows a homogeneous decrease in VWF, with all components present. Patient P.P., besides having a significant decrease in all components, also has unusually large VWF multimers, typical of type Vicenza VWD. Note the significant increase in VWF observed after fainting, together with the appearance of unusually large VWF forms in both patients.**



**Figure 2. Plasma FVIII, VWF:Ag and VWF:RCo before and after fainting episodes in patients C.L. and P.P.**

VWF:Ag content, on the other hand, did not change significantly (mean 77.5 U/dL in stress-free samples vs 83.7 U/dL after fainting). All components of the plasma VWF multimer pattern increased significantly in both patients (Figure 1), with the appearance of extra-large VWF multimers that had not been present before fainting. In patient C.L., who has a normal VWF multimer pattern under resting conditions, fainting induced a pattern similar to that observed after DDAVP infusion, in well-responding patients. In patient P.P., who had unusually large VWF multimers even under resting conditions in accordance with her diagnosis of type Vicenza VWD, fainting induced even higher molecular weight VWF multimers; these multimers, however, were apparently not larger than those observed in the patient C.L. with a quantitative VWF defect. In patient P.P., who fainted during the pre-DDAVP blood sample collection, evaluation of the post-DDAVP plasma VWF concentrations was informative about the post-fainting VWF time-course. In this patient, DDAVP induced an additional increase in FVIII and VWF levels, compared to pre-DDAVP values which, on the other hand, represented the post-fainting situation. At the
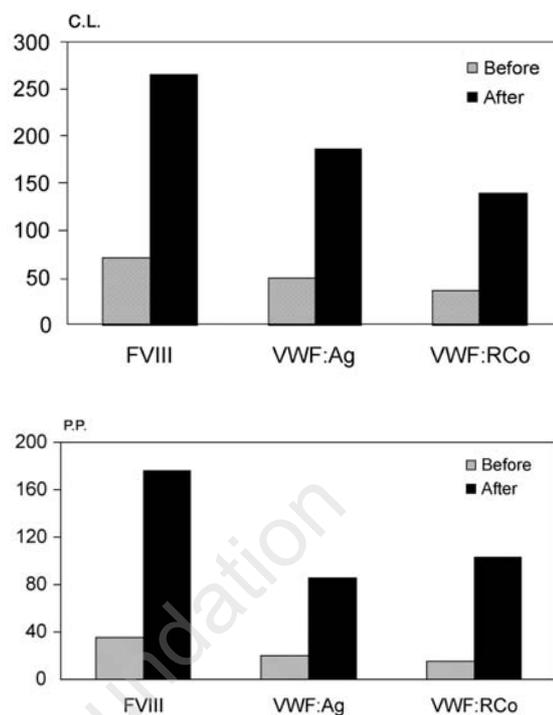
peak, occurring 30 min after DDAVP, VWF:Ag was 173 U/dL, VWF:RCo was 204 U/dL and FVIII was 340 U/dL vs 86.2 U/dL, 103 U/dL and 176 U/dL, respectively, observed before DDAVP. After DDAVP, there were no higher molecular weight VWF multimers than those observed before; indeed, there was a slight disappearance of the higher molecular weight forms starting 30 min after DDAVP, in agreement with the rapid clearance of type Vicenza VWF (Figure 3).[20] All other multimers increased (Figure 3).

## Discussion

We found that fainting induces an acute and significant increase in plasma FVIII and VWF values, together with the appearance in circulation of unusually large VWF multimers, which are normally stored in the cellular compartment. Since the unusually large VWF multimers are hemostatically more efficient, and increased VWF levels are one of the risk factors predisposing to thrombotic complications, we suggest that fainting could be a potential pro-thrombotic condition.

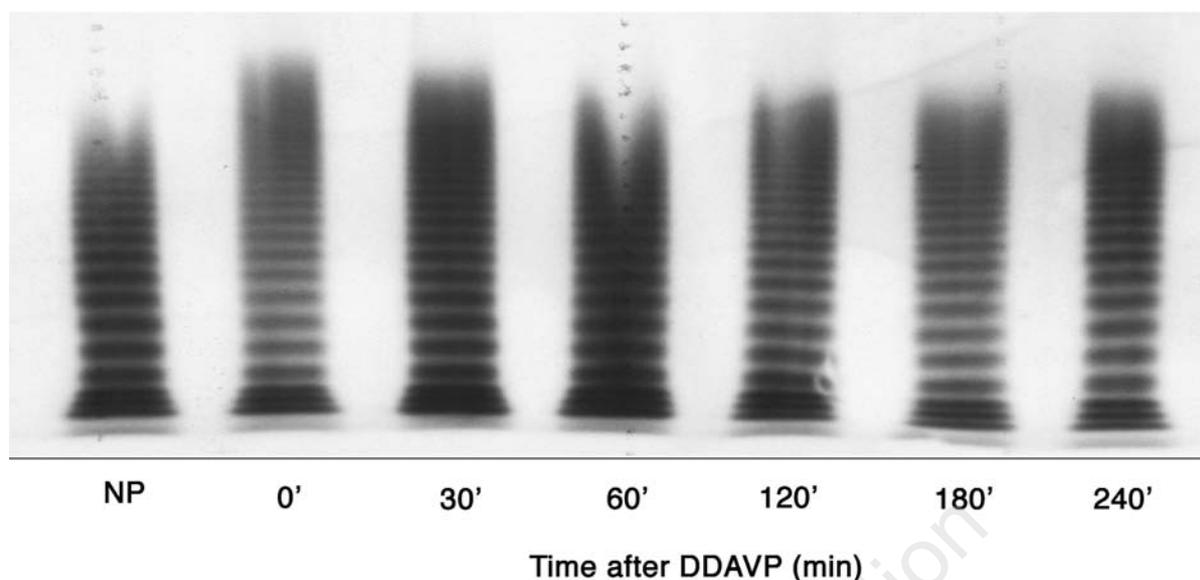Other than in megakaryocytes and platelets, VWF

**Figure 3. VWF multimer pattern before and after DDAVP administration (0.3 µg/Kg) in patient P.P. (type Vicenza VWD). Time 0 represents, simultaneously,  the pre-DDAVP sample, since it was collected before DDAVP administration, and the post-fainting  blood sample, because it was collected after the patient had fainted.**

is also stored in the Weibel–Palade bodies of endothelial cells, these organelles being the main storage site of extra–large VWF multimers.[21] VWF may be mobilized from these organelles and released into the circulation in response to secretion stimuli, and in circumstances that require an efficient coagulation system, such as those occurring at the site of vascular injury. Many different secretion stimuli have been identified, e.g. thrombin, adrenaline, histamine and the vasopressin analog DDAVP.[14] The VWF acutely secreted by endothelial cells is characterized by the presence of large VWF multimers that are normally not present in circulation. VWF is a large glycoprotein, indeed the largest soluble molecule found in humans, and is arranged in polymers (multimers) of increasing size from $500 \times 10^3$ up to 20 million Daltons. This heterogeneous structure assures modulation of VWF function because large forms are more efficient in adhering to the subendothelial matrix, are known to possess higher affinity for platelet GPIb and are, therefore, more capable of promoting the formation of platelet aggregates.[22,23] Unusually large VWF multimers are also able to induce circulating platelet aggregates, as in thrombotic thrombocytopenic purpura, where they induce the formation of a hyaline platelet plug in the microcirculation.[24] The largest VWF multimers have also been shown to aggregate platelets under high shear stress conditions, such as those occurring in stenotic arteries.[25] Furthermore, VWF seems to play a role in

thrombosis when the levels of this factor are increased. Patients with cardiovascular diseases have been observed to have major clinical events, such as death and myocardial infarction, in the presence of high plasma levels of VWF.[26] On the other hand, the absence of large VWF multimers predisposes to bleeding.[27]

In our patients, fainting was associated with acute hypotension and was caused by the fear of venipuncture and the sight of blood, e.g. potentially stressful conditions. Both an adrenergic stimulus and vasodilatation might have been involved in this acute release of VWF. Indeed, it was demonstrated that adrenaline could act as a secretory agent *in vitro* and release VWF from endothelial cells.[28] Whether adrenaline alone is responsible for the VWF release or whether its action is increased by the acute fall in blood pressure remains to be clarified. Similarly, it is not clear whether, and if so, how vasodilating shock molecules are involved. Blood pressure is known to modulate the amount of plasma VWF and patients with hypertension have been demonstrated to have increased VWF levels.[29] However, this increase is most probably the consequence of an upregulation of the constitutive release of VWF from endothelial cells, rather than an acute release of VWF. Nevertheless, it cannot be excluded that a sudden blood pressure modification, evolving into hypertension or hypotension, may activate the acute release of VWF from endothelial cells. Regardless of its etiopathogene-

sis, fainting was associated with a significant increase in VWF levels in VWD patients with low or very low plasma levels of VWF. The FVIII and VWF increases appeared to range from 4 to 5 times the levels at resting conditions, so that their values overshot the upper limit of the normal range. This event was also associated with a drastic modification of the VWF multimer pattern; as well as an increase in each single oligomer, unusually large VWF multimers, normally confined to cellular compartments (platelets and endothelial cells), were also present, resembling the findings when VWF is acutely released by DDAVP. Which cellular granules release VWF ? Platelet α-granules, endothelial cell Weibel-Palade bodies, or both? Even though platelet activation is accompanied by VWF release, it is known that after release, VWF is bound to platelet GPIb and does not appear in the soluble phase. Hence, it is likely that the VWF release induced by fainting is attributable to endothelial cells alone. The acute release of VWF did not induce its complete depletion from the Weibel-Palade bodies, because a successive secretion stimulus, such as DDAVP, induced an additional release of VWF. Indeed, no additional secretion of abnormally large VWF multimers was observed, suggesting that these large forms are maximally secreted by fainting, which therefore acts as a true secretion stimulus.

Vasodilation can be considered a primary defense mechanism just as the fainting-induced increase in plasma VWF is suggestive of *defensive activation* of endothelial cells. From this point of view, vasodilation and VWF release, whatever their chronological sequence or cause-effect relationship, should be considered part of the emergency response to stress. It is possible that this hemostatic condition in an already compromised cardiovascular system, and in the presence of other risk factors, such as low VWF-cleaving protease[30] and/or high levels of VWF,[26] may favor an ongoing thrombotic complication. This deserves further investigation but the entity of the phenomenon suggests that fainting may tentatively be included among pro-thrombotic conditions.

Finally, from another point of view, we must consider fainting, or conditions of stress in general, as factors that may affect the diagnosis of VWD bacause of the changes in plasma VWF levels. This observation may also explain the low penetrance of VWD and the variable phenotypic expression of this disorder.[31]

In conclusion, we have identified fainting as a new stimulus causing the release of VWF from endothelial cells. This observation opens new horizons regarding the possible pathophysiology of the thrombotic complications that may develop during stressful conditions, particularly if these conditions are persistent and/or repeated.

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## Pre-publication Report & Outcomes of Peer Review

### Manuscript processing

This manuscript was peer-reviewed by two exter-nal referees and by Professor Vicente Vicente, Deputy Editor. The final decision to accept this paper for pub-lication was taken jointly by Professor Vicente and the Editors. Manuscript received January 21, 2003; accepted March 23, 2003.

In the following paragraphs, Professor Vicente summarizes the peer-review process and its out-comes.

### What is already known on this topic

Many different stimuli, such as adrenaline, hista-mine, thrombin and vasopressin analogs are respon-sible for increases in FVIII and vWF plasma levels.

### What this study adds

Two clinical observations in von Willebrand patients indicate that fainting is a new stimulus that is able to induce an acute and significant increase in FVIII and vWF plasma proteins.