

Prolactin as a modulator of platelet function and thrombosis: The end of the story, or a new beginning?

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Publication of negative results, i.e. those showing complete lack of a presumed effect, remains an exception in biomedical journals. This is particularly true for experimental studies which do not need to be pre-registered like clinical trials. In this issue of *Thrombosis and Haemostasis*, Reuwer et al. present the findings of a systematically performed study which addressed the effects of the hormone prolactin on human platelets (1). In this regard, a series of previous publications, all by one group, had consistently reported that prolactin is a platelet agonist, and suggested that its effects on platelet function might partly explain the increased occurrence of thrombotic events in pregnancy, in patients with pituitary tumours or those receiving anti-psychotic drugs, and even in association with ischaemic stroke or acute coronary syndromes (2–5). Reuwer et al. now report that, in their studies, a) prolactin was neither by itself nor in combination with adenosine diphosphate (ADP) capable of enhancing platelet aggregation; b) prolactin did not affect platelet activation as assessed by P-selectin expression or PAC-1 binding on flow cytometry; and c) neither resting nor maximally activated platelets expressed detectable levels of the prolactin receptor. Indeed, the results could not be more negative. Is this then the end of the prolactin story?

Over the past years, there has been increasing awareness of the importance of platelet activation for the pathogenesis of atherothrombosis (6, 7). The platelet activation process is initiated by platelet adhesion to von Willebrand factor, collagen, and other extracellular matrix proteins (fibronectin, vitronectin, and laminin); it involves the synergistic effects of thrombin, thromboxane A₂, ADP, epinephrine, and serotonin (5-hydroxytryptamine) acting on stimulating receptors coupled to G proteins (8). However, apart from the “classical” inducers and pathways of platelet activation, hormones associated with cardiovascular risk factors have directly been implicated in the modulation of platelet function and thrombosis (9). More specifically, at the beginning of this decade we and other investigators focussed on the possible prothrombotic role of the adipocytokine leptin which is elevated in the circulation of almost all obese individuals. In-vivo studies using leptin-deficient and leptin receptor (ObR)-deficient mice indicated that leptin specifically enhances thrombus formation

following experimental arterial injury (10, 11). In further studies, leptin also appeared to promote experimental venous thromboembolism (12). The prothrombotic effects of the hormone apparently were mediated by a receptor-specific enhancement of platelet activation (13–15), suggesting that leptin may be a (weak) platelet agonist. Thus, assuming the absence of “peripheral” (platelet) resistance to leptin in obesity (16), the hyperleptinaemia encountered in this condition might provide a direct link between excess body weight and cardiovascular risk. Of note, adiponectin which is generally considered an atheroprotective adipocytokine, was recently reported to have opposing, i.e. inhibitory effects on platelet activation and thrombus formation following vascular injury (17).

Could prolactin be yet another hormone linking arterial and possibly venous thrombosis to certain pathologic conditions? Like ObR, the prolactin receptor (PRL-R) also belongs to the class I cytokine receptor family (18), and an early publication by Wallaschowski et al. suggested the presence of the short form of PRL-R on human platelets (5). Although the intracellular signalling pathways of prolactin were not further dissected in that study, the authors reported activation of protein kinase C, a molecule which lies downstream of both phospholipase (PL)C β and PLC γ and might thus represent a distal common pathway of G-coupled activation receptors and ObR as well (8, 13, 15). These observations appeared to agree with the enhancement, by prolactin, of ADP-induced platelet aggregation and with the expression of P-selectin, the latter serving as a surrogate marker for platelet activation (5, 19). Combined with preliminary clinical data by the same authors, it became increasingly plausible that hyperprolactinaemia, such as encountered in pregnancy and other conditions including some cases of obesity, might contribute to the pathogenesis of arterial and venous thrombosis (4). However, this interesting hypothesis was first challenged by Atmaka et al. who failed to find an association between hyperprolactinaemia and enhanced platelet aggregation in pregnant women (20). Now, in a thorough investigation, Reuwer et al. contradict, and actually deconstruct, all the experimental evidence supporting the prolactin story (1). Neither could PRL-R be detected on human platelets, nor did prolactin increase platelet

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activation or aggregation *in vitro*. The authors provide a number of possible technical explanations for the discrepancy between their results and those reported previously. Of these, the use of different antibodies against PRL-R in the present as opposed to the previous (5) study may be capable of explaining, at least in part, the diverging findings of the Western blot analysis in the two publications and also the negative findings of the flow cytometry studies of Reuwer et al. On the other hand, the contradicting results of the aggregation studies remain almost impossible to reconcile.

What conclusions can be drawn from the prolactin debate? On the one hand, it again becomes clear that a) clinical associations between the circulating levels of a hormone and the occurrence of thrombotic events in small or moderately sized patient groups and b) *in-vitro* platelet studies showing platelet aggregation or expression of activation markers after stimulation with this particular hormone, should always be interpreted with caution and regarded as hypothesis-generating. Clearly, the definitive proof of a relevant, platelet-specific pathophysiological mechanism also requires the clear demonstration of the receptor and the description of the signaling pathway(s) of this hormone in human platelets. This latter step may often be more complex and cumbersome than originally anticipated. In the case of leptin

for example, the presence of the long, signalling form of ObR on human platelets was reported on megakaryocytes (21) and platelets (22) more than 10 years ago, and subsequently supported by both *in-vivo* (10, 11) and *in-vitro* data (13, 15). However, the affinity of platelet ObR for leptin was subsequently found to be very low (with a *Kd* of 76–158 nM) (23), and very recent data obtained in megakaryocytes suggest that these cells express only the short isoform of ObR in two distinctly glycosylated forms (24). Although the short isoform of ObR is capable of binding leptin with high affinity, its signalling capacity and intracellular pathways remain to be elucidated (25).

On the other hand, and more importantly, the prothrombotic effects of a hormone may involve cell types other than platelets. In this regard, two recent publications showed that leptin may induce the expression of tissue factor by human neutrophils and mononuclear cells (26, 27), thus suggesting a novel possible link between obesity, inflammation, and thrombosis. Interestingly, in their publication Reuwer et al. discuss the evidence suggesting that prolactin may also affect thrombosis by stimulating inflammatory cells (1). This exciting potential mechanism deserves to be investigated further and may eventually turn out to be the true legacy of the prolactin debate.

References

1. Reuwer AQ, Nieuwland R, Fernandez I, et al. Prolactin does not affect human platelet aggregation or secretion. *Thromb Haemost* 2009; 101: 1119–1127.
2. Raaz D, Wallaschofski H, Stumpf C, et al. Increased prolactin in acute coronary syndromes as putative Co-activator of ADP-stimulated P-selectin expression. *Horm Metab Res* 2006; 38: 767–772.
3. Wallaschofski H, Lohmann T, Hild E, et al. Enhanced platelet activation by prolactin in patients with ischemic stroke. *Thromb Haemost* 2006; 96: 38–44.
4. Wallaschofski H, Kobsar A, Sokolova O, et al. Co-activation of platelets by prolactin or leptin—pathophysiological findings and clinical implications. *Horm Metab Res* 2004; 36: 1–6.
5. Wallaschofski H, Kobsar A, Koksich M, et al. Prolactin receptor signaling during platelet activation. *Horm Metab Res* 2003; 35: 228–235.
6. Davi G, Patrono C. Platelet activation and atherothrombosis. *N Engl J Med* 2007; 357: 2482–2494.
7. Ruggeri ZM. Platelets in atherothrombosis. *Nat Med* 2002; 8: 1227–1234.
8. Varga-Szabo D, Pleines I, Nieswandt B. Cell adhesion mechanisms in platelets. *Arterioscler Thromb Vasc Biol* 2008; 28: 403–412.
9. Bodary PF. Links between adipose tissue and thrombosis in the mouse. *Arterioscler Thromb Vasc Biol* 2007; 27: 2284–2291.
10. Bodary PF, Westrick RJ, Wickenheiser KJ, et al. Effect of leptin on arterial thrombosis following vascular injury in mice. *Jama-Journal of the American Medical Association* 2002; 287: 1706–1709.
11. Konstantinides S, Schäfer K, Koschnick S, et al. Leptin-dependent platelet aggregation and arterial thrombosis suggests a mechanism for atherothrombotic disease in obesity. *J Clin Invest* 2001; 108:1533–40.
12. Konstantinides S, Schäfer K, Neels JG, et al. Inhibition of endogenous leptin protects mice from arterial and venous thrombosis. *Arterioscler Thromb Vasc Biol* 2004; 24: 2196–2201.
13. Dellas C, Schäfer K, Rohm IK, et al. Leptin signaling and leptin-mediated activation of human platelets: importance of JAK2 and the phospholipases Cgamma2 and A2. *Thromb Haemost* 2007; 98: 1063–1071.
14. Elbatarny HS, Maurice DH. Leptin-mediated activation of human platelets: involvement of a leptin receptor and phosphodiesterase 3A-containing cellular signaling complex. *Am J Physiol Endocrinol Metab* 2005; 289: E695–E702.
15. Corsonello A, Malara A, De Domenico D, et al. Identifying pathways involved in leptin-dependent aggregation of human platelets. *Int J Obes Relat Metab Disord* 2004; 28: 979–984.
16. Dellas C, Schäfer K, Rohm I, et al. Absence of leptin resistance in platelets from morbidly obese individuals may contribute to the increased thrombosis risk in obesity. *Thromb Haemost* 2008; 100: 1123–1129.
17. Kato H, Kashiwagi H, Shiraga M, et al. Adiponectin acts as an endogenous antithrombotic factor. *Arterioscler Thromb Vasc Biol* 2006; 26: 224–22430.
18. Zabeau L, Lavens D, Peelman F, et al. The ins and outs of leptin receptor activation. *FEBS Lett* 2003; 546: 45–50.
19. Wallaschofski H, Kobsar A, Sokolova O, et al. Differences in platelet activation by prolactin and leptin. *Horm Metab Res* 2004; 36: 453–457.
20. Atmaca A, Gurlek A, Dagdelen S, et al. Hyperprolactinemia of pregnancy is not associated with increased *in vivo* platelet activity and shortened *in vitro* bleeding times. *Exp Clin Endocrinol Diabetes* 2006; 114: 188–191.
21. Gainsford T, Willson TA, Metcalf D, et al. Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells. *Proc Natl Acad Sci USA* 1996; 93: 14564–14568.
22. Nakata M, Yada T, Soejima N, et al. Leptin promotes aggregation of human platelets via the long form of its receptor. *Diabetes* 1999; 48: 426–429.
23. Giandomenico G, Dellas C, Czekay RP, et al. The leptin receptor system of human platelets. *J Thromb Haemost* 2005; 3: 1042–1049.
24. Kamikubo Y, Dellas C, Loskutoff DJ, et al. Contribution of leptin receptor N-linked glycans to leptin binding. *Biochem J* 2008; 410: 595–604.
25. Bjorbaek C, Kahn BB. Leptin signaling in the central nervous system and the periphery. *Recent Prog Horm Res* 2004; 59: 305–331.
26. Rafail S, Ritis K, Schäfer K, et al. Leptin induces the expression of functional tissue factor in human neutrophils and peripheral blood mononuclear cells through JAK2-dependent mechanisms and TNFalpha involvement. *Thromb Res* 2008; 122: 366–375.
27. Napoleone E, DI SA, Amore C, et al. Leptin induces tissue factor expression in human peripheral blood mononuclear cells: a possible link between obesity and cardiovascular risk? *J Thromb Haemost* 2007; 5: 1462–1468.