

## ORIGINAL ARTICLE

# Matrix Metalloproteases and Cathepsin D in Human Serum do not Cleave Prolactin to Generate Vasoinhibin

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### SUMMARY

**Background:** Vasoinhibin is generated in the pituitary gland and in multiple target tissues by proteolytic cleavage of prolactin by matrix metalloproteinases and cathepsin D. A dysregulation of vasoinhibin generation appears to contribute to diabetic retinopathy and diabetic macular edema, retinopathy of prematurity, peripartum cardiomyopathy, and preeclampsia. Here, we investigate whether vasoinhibin is generated by matrix metalloproteinases and cathepsin D in human serum.

**Methods:** The abundance of matrix metalloproteinases 1, 2, 3, 8, 9, 10, 13, tissue inhibitors of metalloproteinases 1, 2, 4, and the activity of cathepsin D in serum samples were determined. Samples from healthy male (n = 3) and female (n = 2) subjects, pregnant subjects (n = 2), and patients with type 2 diabetes mellitus (n = 2) were investigated. The samples were incubated with recombinant prolactin at 37°C, under different pH, time, and buffer conditions. Prolactin and cleaved prolactin products were investigated by SDS-PAGE and western blotting.

**Results:** Matrix metalloproteinases-1, -2, -3, -8, -9, -10, -13, TIMP-1, -2, and -4, and the activity of cathepsin D were detected in all sera. Full-length prolactin incubated with human sera, containing endogenous matrix metalloproteinases and cathepsin D, remained intact at neutral pH during a time frame from 1 to 24 hours. Partial enzymatic cleavage of prolactin resulting in the generation of a vasoinhibin-like 17 kDa peptide was observed in samples incubated at pH 3.4. Heat inactivation of the serum and the addition of an MMP inhibitor suppressed the generation of the 17 kDa peptide, indicating that its generation was MMP-mediated.

**Conclusions:** Vasoinhibin generation by enzymatic cleavage of prolactin by matrix metalloproteinases or cathepsin D does not occur in human serum at physiological pH. A limited proteolysis of prolactin, resulting in the generation of a vasoinhibin-like peptide with an apparent molecular weight of 17 kDa occurs in serum at acidic pH. The generation of vasoinhibin may require the cellular and tissue microenvironments.

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Supplementary Figures

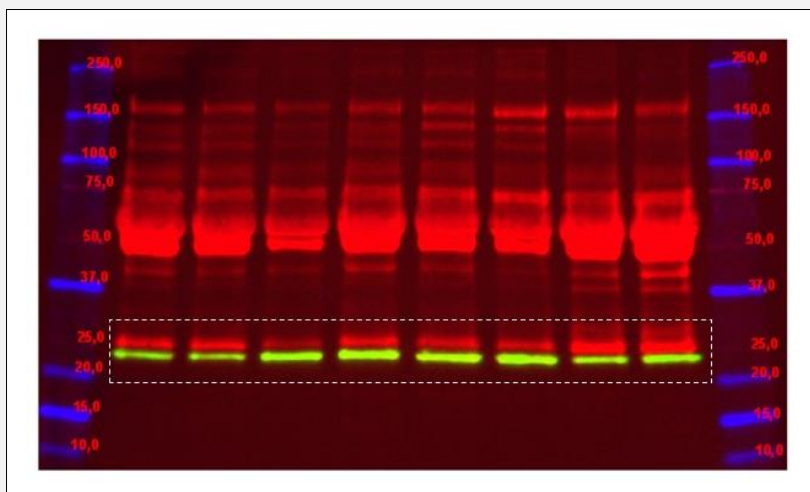


Figure S1.

Whole-membrane, multichannel-image of serum incubated with recombinant PRL at pH 7.4, corresponding to Figure 4A. Total protein is shown in red (UV-activated total protein staining of stain-free gel), molecular weight markers (left and right lanes) are blue and red, and chemiluminescence signals of the western blot are green, demonstrating intact PRL at 23 kDa. The area shown in Figure 4A is marked by the dotted square.

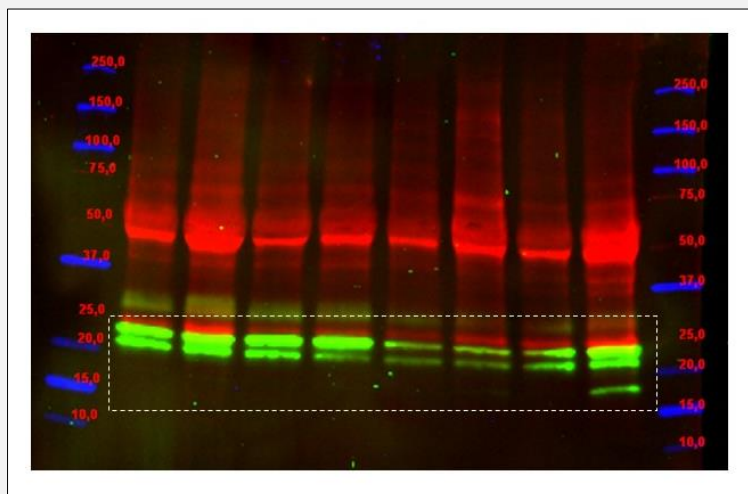


Figure S2.

Whole-membrane, multichannel-image of serum incubated with recombinant PRL at pH 3.4, corresponding to Figure 4B. Total protein is shown in red (UV-activated total protein staining of stain-free gel), molecular weight markers (left and right lanes) are blue and red, and chemiluminescence signals of the western blot are green, demonstrating intact PRL at 23 kDa and a cleaved 17 kDa PRL-fragment (right). The area shown in Figure 4B is marked by the dotted square. A slight immunoreactive signal can be seen at approximately 28 kDa, the identity of the serum protein responsible for this immunoreactivity is not known.