

Effects of Vitamin D on endothelial-derived factors implicated in the atheromatic plaque vulnerability

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1 AIM OF THE STUDY

Although the protective effects of $1,25(\text{OH})_2\text{D}_3$ (Vit D) on the early stages of atheromatosis process have been investigated, data on its influence on factors implicated in plaque vulnerability are limited. Among the key vessel wall component in the later stages of atherogenic process is endothelium. During the stages of plaque rupture and/or erosion metalloproteinases MMP-2, MMP-9, their inhibitors TIMP-1, TIMP-2, the RANKL-RANK-OPG system and MCP-1 which all expressed by endothelial cells, play a critical role. Herein, we aimed to investigate the effect of vitamin D on the expression of the above factors on endothelial cells.

2 MATERIALS AND METHODS

➤ Human aortic endothelial cells (HAECs) were incubated with VitD for 24, 48, 72 hours at a concentration range 10^{-8} – 10^{-11} in the absence of TNF- α or with Vit D for 24 hours in the presence of TNF- α at concentration 10ng/ml for the last 6 hours.

➤ Real Time-PCR was used to quantitatively detect gene expression of MMP-2, MMP-9, TIMP-1, TIMP-2, OPG, RANKL, RANK and MCP-1.

➤ Gelatin zymography was used to detect MMP-2, MMP-9 activities in culture supernatants.

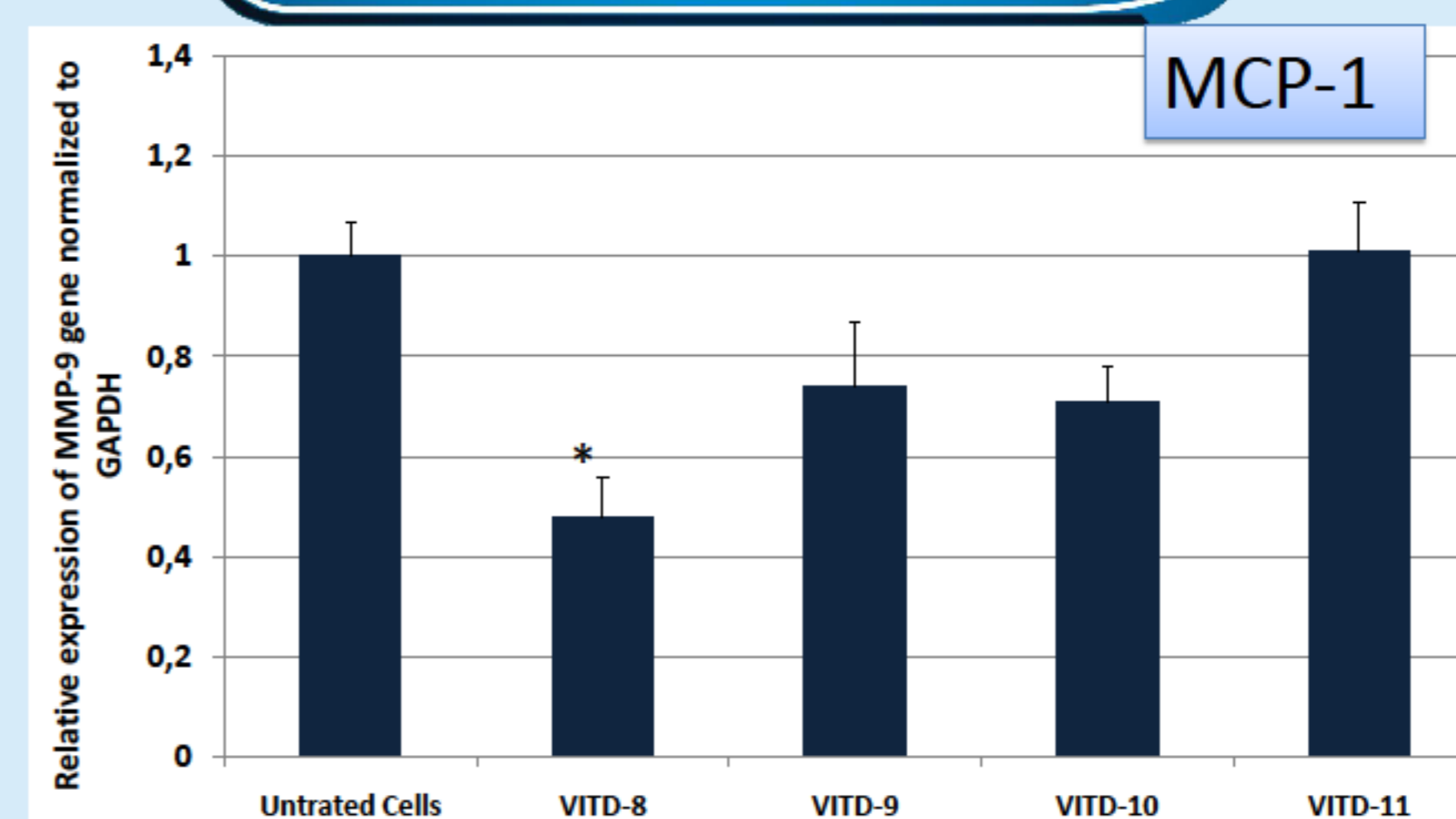
➤ OPG protein levels were also evaluated by western blot.

➤ Our results are expressed as mean SD. Two-tailed Student's test was used for our statistical analysis and p value <0.05 was considered significant.

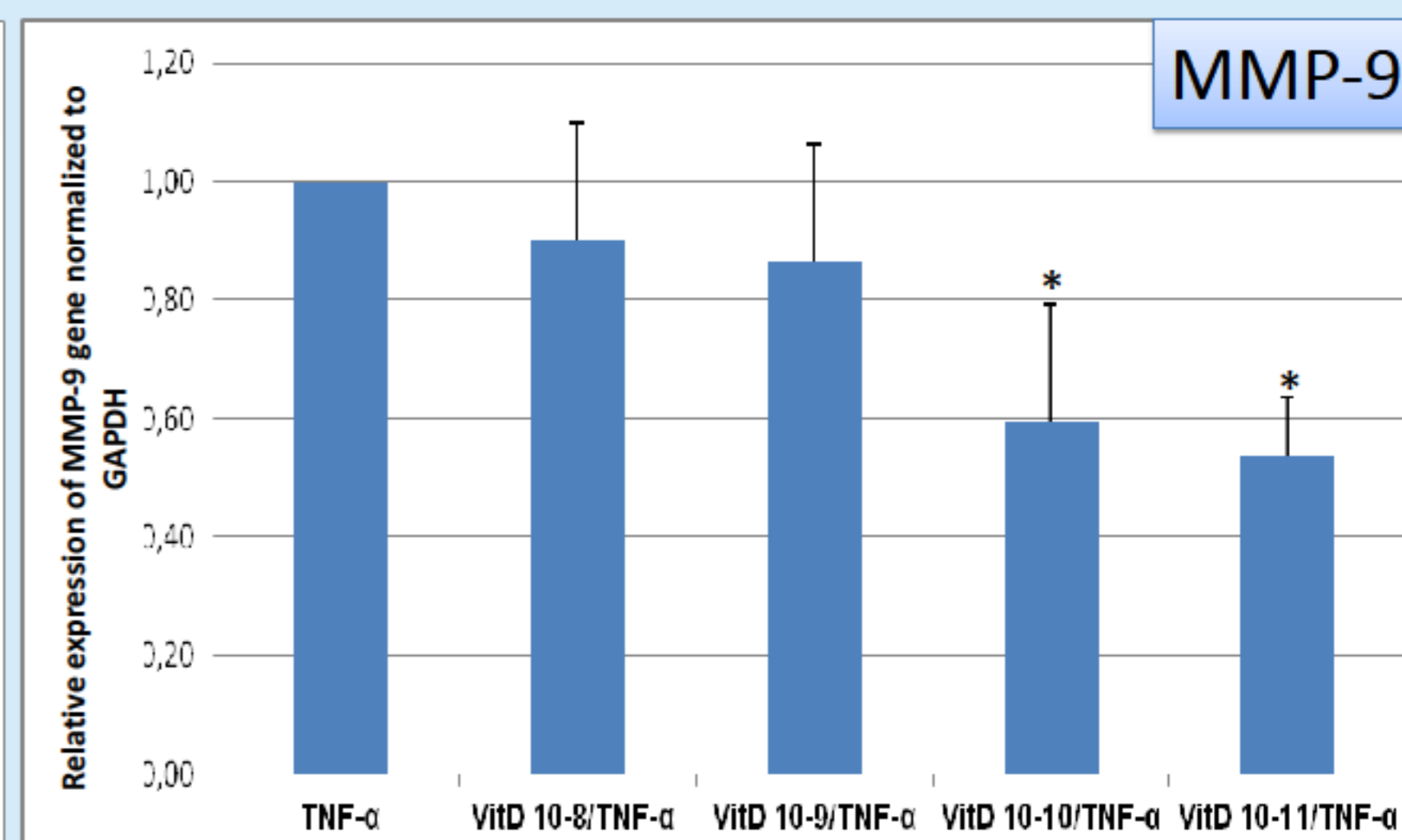
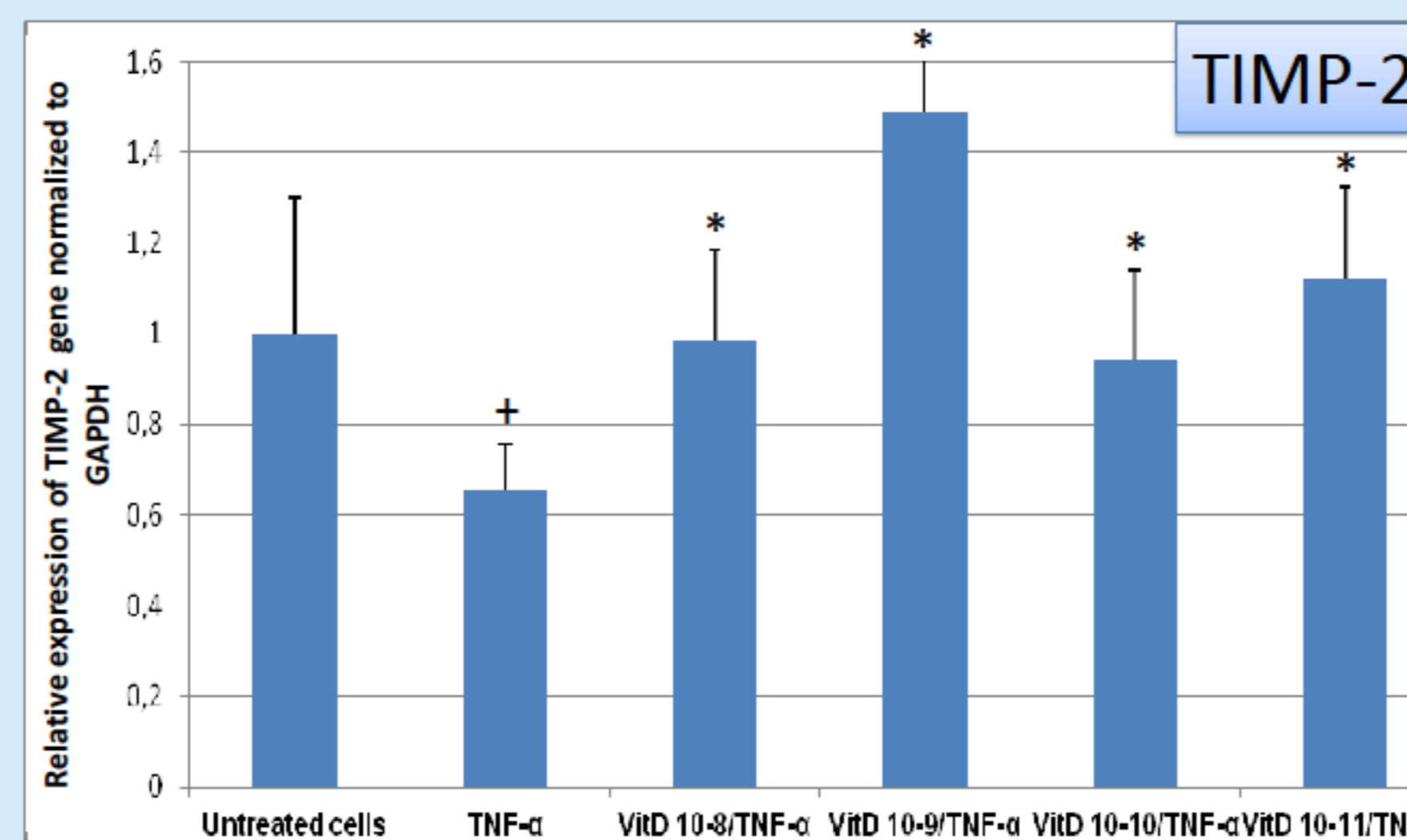
3 Results

- ❖ VitD did not significantly change the expression of MMP-9, MMP-2, TIMP-1 and TIMP-2 at mRNA level at various concentrations and incubation time-courses, however, decreased the expression of *MCP-1* dose-dependently.
- ❖ The expression of OPG at mRNA was significantly increased in the presence of TNF- α and this effect became more pronounced when cells were preincubated with Vit D at concentration 10^{-9} M. However, our results were not confirmed at protein level. Interestingly, incubation with p38 inhibitor, totally abolished the effect of Vit D on the mRNA OPG expression, indicating that this MAPK pathway is implicated in the regulation of OPG expression by Vit D.
- ❖ When cells activated with TNF- α , preincubation with Vit D resulted in a significant decrease in *MMP-9* expression at 10^{-10} and 10^{-11} M and increase in *TIMP-2* expression while it didn't affect the expression of *MMP-2* and *TIMP-1*.
- ❖ Gelatin zymography revealed that Vit D decrease the active MMP-2 dose-dependently with the effects being more pronounced at the lower concentrations.

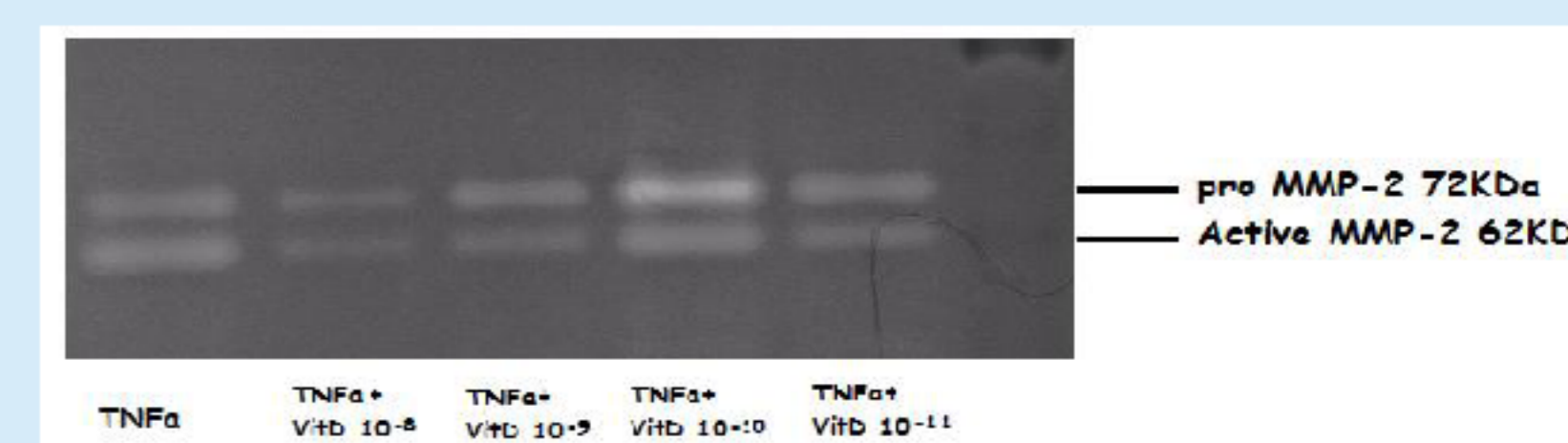
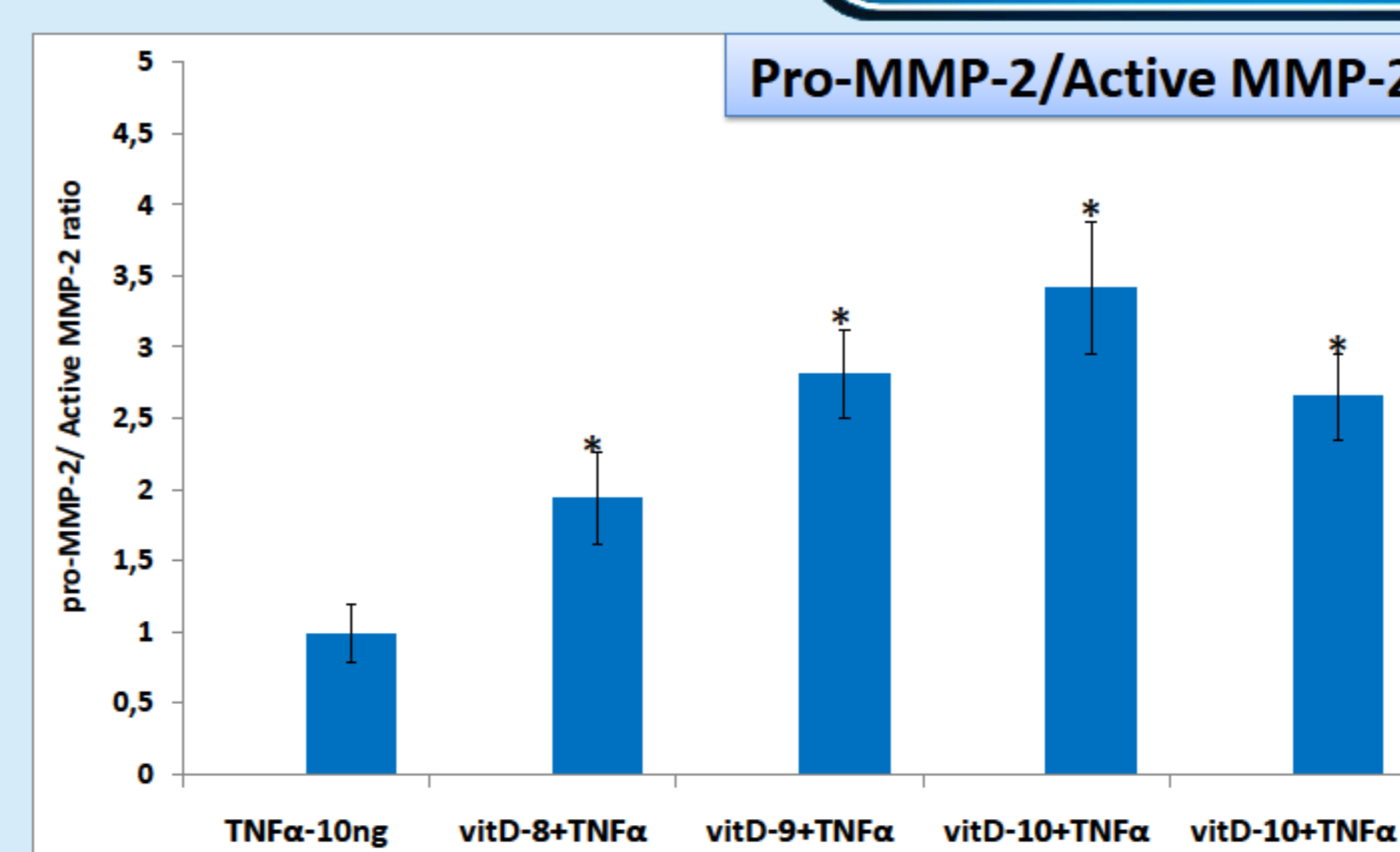
Real time PCR



➤ The statistically significant reduction in TNF- α compared to control is marked with + (p < 0,05), and the statistically significant increase of the samples in relation to TNF- α is marked with * (* p < 0,05 and ** p < 0,01)



Gelatin Zymography



4 Conclusions

In the presence of TNF α (resembling the low grade inflammation state of established atherosclerosis) $1,25(\text{OH})_2\text{D}_3$ at physiologically achievable concentrations reduces the expression of *MMP-9* and induces the expression of *TIMP-2* at mRNA level implying a possible protective role in the later stages of atheromatosis, such as plaque vulnerability. Further studies are needed to elucidate the underlying mechanisms.

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