Lipoproteins and their modified forms regulate early stage vascular smooth muscle cell calcification

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INTRODUCTION

Vascular calcification (VC) is a strong, independent risk factor for cardiovascular events and is the active process of vascular smooth muscle cell (VSMC) differentiation into bone-like mineralising cells. Because of the colocalisation of VC with atherosclerosis, we hypothesise that lipoproteins might have a role in the progression of this disease.

Runx2: Runt-related transcription factor 2;
RANK: Receptor activator of NF-κB;
RANKL: RANK ligand; ALP: Alkaline Phosphatase; OPG: Osteoprotegerin.

SUMMARY

CONCLUSION

- Atherogenic lipoproteins stimulate VC *in vitro*
- HDL inhibits VC progression *in vitro* and *in vivo***
- Lipoproteins effect the expression of Runx2 and OPG mRNA

In continuing this study, we aim to further identify the mechanisms behind these interactions, potentially identifying a target for a therapeutic to VC.

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METHODS

rHDL inhibits vascular calcification by reducing pro-calcification gene expression *in vitro*

Figure 1: HAoSMC were incubated for 15 days with CM after pre-treatment with lipoproteins and harvested for VC quantification. Calcification was detected using an alizarin red s (ARS) stain assay. Representative images are shown above corresponding bar. Data is represented as fold change of calcium phosphate (CP) only controls, mean ± SEM; student's t-tests (n=3), *p<0.05, **p<0.01, ****p<0.0001.

Figure 2: HAoSMC were incubated for 7 days with CM after pre-treatment with rHDL and harvested for qPCR mRNA detection. Data is normalized to GAPDH and represented as percentage expression of no treatment controls, mean ± SEM; ANOVA (n=3), *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

rHDL inhibits early stage microcalcification progression and stimulates late stage macrocalcifications *in vivo*

Figure 3: Formalin fixed, paraffin embedded brachiocephalic (BCA) artery (3A,3B) and aortic leaflet (3C) sections of Apo E^{-/-} and OPG^{-/-} Apo E^{-/-} mice were stained with H&E (3A) or alizarin red s stain (3B,3C). Percentage area of plaque (3A) or plaque calcification (3B,3C) was measured and analysed using a 2-way ANOVA between strains and t-test between treatments per strain (n=3-10). * p<0.05, ** p<0.01, ****p<0.0001. Representative images are shown below their respective bar graphs.