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## Acetylated LDL induces IL-1 $\beta$ release from Human Coronary Endothelial Cells

**Title:** Acetylated LDL induces IL-1 $\beta$  release from Human Coronary Endothelial Cells

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**Background:** Atherosclerosis is a chronic vascular inflammatory disease characterised by disturbed arterial blood flow due to the atheromatous plaque build-up within arterial layers. Lipid rich plaques contain various forms of cholesterol, such as acetylated low density lipoprotein (AcLDL), that undergo many modification processes and which enter vascular cells, initiating over exuberant repair processes leading to arterial occlusion and life threatening myocardial infarction or stroke. Endothelial cells (EC) strongly drive the pathogenesis of atherosclerosis by producing pro-inflammatory cytokines, such as interleukin-1 beta (IL-1 $\beta$ ). We have recently shown that the release of IL-1 $\beta$  from EC occurs via extracellular lysosomal derived vesicles, but the stimuli and mechanisms by which IL-1 $\beta$  is released remain to be fully elucidated. We hypothesise that AcLDL enters the arterial endothelium and induces IL-1 secretion, potentially via a caspase-1/NLRP3 mechanism.

**Methods:** Human coronary artery endothelial cells (HCAEC) isolated from three different donors were cultured and stimulated with pro-inflammatory cytokines TNF $\alpha$  and IL-1 $\alpha$  (10 ng/ml each, for 48 hours), followed by incubation with native human AcLDL cholesterol at multiple concentrations (10-200 ug/ml) for 6 hours. Cell lysates and culture supernatants were collected and analysed for IL-1 $\beta$  using ELISA. Cell viability was also measured.

**Results:** AcLDL induced the release of IL-1 $\beta$  from stimulated HCAECs in a dose dependent manner with maximum release (155.4 pg/ml, n=3) at concentration of 50 ug/ml. This was 4 fold greater than that released by cytokine-stimulated (38.49 pg/ml, n=3) and 3 fold greater than that released by neutrophil elastase (49.45 pg/ml, n=3). This release was not caused by toxicity of the AcLDL: cell viability was confirmed by lactate dehydrogenase cell viability assay.

**Conclusion:** AcLDL is capable of eliciting IL-1 $\beta$  release in activated HCAECs, without causing toxicity.